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ERRATA AND AUTHORS' EMENDATIONS

Page 178, line 6, "are presented" should be "are not presented."

Page 236, the curve in figure 3 should contain only five points, as indicated in text.

Page 274, line 2, before "Lucette" insert "Alice Tiplady,".

Page 301, figure 11, omit black dot at right in *A* and *C*.

Page 309, figure 13, new drawing furnished to correct errors in *A* and *C*.

Page 336, last line under "Discussion", insert "no" before "appreciable."

Page 429, sixth line from bottom, "2.26±0.04" should be "5.00±0.05" and "5.00±0.05" should be "2.26±0.04."

Page 513, table 3, item 7, "(413.33390.75)" should be "(413.33 390.75)."

Page 712, table 4, last column, sample 17, "8.82" should be "3.82."

Page 719, table 6, last column, average of samples 13 and 14 (fresh weight), ".073" should be ".079."

Page 732, table 11, next to last column, average of samples 7 and 8, "3.33" should be "2.33."

Page 737, seventh line from bottom, "vasal" should be "basal."

Page 794, eighth line from bottom, "no. 5" should be "no. 6."

Page 799, table 4, column 1, third line from bottom, and column 3, bottom line, "*Z. mays* (F$_2$)" should b "*Z. mays* × (F$_2$)."

Page 812, sixteenth line from bottom, "nor in agar tubes" should be "and no growth occurred in shak agar tubes."

Page 830, footnote 4, after last equality sign, "$_0$.9692" should be "0.9692."

Page 840, table 2, column 4, reaction for fowls 74, 10, and 90 should be shown as positive.

Page 918, legend for figure 1, asterisk (*) should be "°".

Page 1011, first line, omit sentence beginning "Although a few points."

Page 1105, under "Beef-infusion agar slants", second and third sentences should be combined to read "Under the hand lens it has a metallic luster on beef agar, pH 6.8, at temperatures of 25° to 30° C."

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CORRELATIONS BETWEEN SEVERITY OF PRUNING AND SUBSEQUENT GROWTH AND FRUIT YIELD OF APRICOT TREES [1]

By H. S. REED [2]

Plant physiologist, California Agricultural Experiment Station

INTRODUCTION

The question often asked, "Does severe pruning really benefit the fruit tree?" has seldom been answered definitely, although scores of experiments have been undertaken with the expectation of getting information on this point. Many of these experiments were begun with young trees and were terminated before enough time had elapsed to make the tests really valid. Young trees, free from serious competition in the root zone, usually grow and produce good fruit without much reference to the system of pruning employed. The important question is the effect of pruning when in later years competition becomes severe between the trees both above and below the surface of the soil.

Another class of experiments has been seriously handicapped by the conditions resulting from a period of rather free growth due to a lack of pruning before the experiment was initiated. The profound disturbance of the physiological balance of trees that were heavily pruned after having made unrestricted growth introduced a large, but indeterminate, variable into the problem.

This paper contains an analysis of the pruning results based largely upon the coefficients of gross and partial correlation between the amount of wood removed, the growth of the tree, the amount of fruit produced, and the average number of fruits per pound. The records were obtained from an orchard of 280 Royal apricot trees on which the writer conducted experiments in pruning for 16 years.

PLAN OF THE EXPERIMENTS

The orchard consisted of 56 plots in 4 replications of 14 plots each, planted for the pruning experiment. Each replicated plot contained 5 trees. The arrangement of these plots, to which 10 systems of pruning were applied, is shown in figure 1. Plots 1 to 4 were pruned both in the summer and in the winter; plots 5 to 10 were pruned in the winter only. The types of pruning and the plots on which they were applied were as follows:

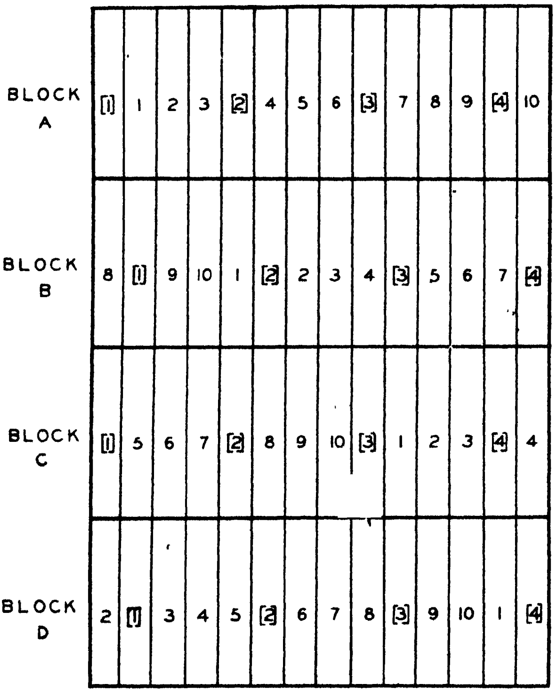
---

[1] Received for publication June 10, 1933; issued February 1934. Paper no. 284, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station

[2] Grateful mention is made of the assistance of J. Enderud in conducting the experiments and preparing the illustrations for this paper, and also of that of Hannah D. Reed in computing most of the coefficients of correlation and in compiling data

Plots pruned summer and wi[illegible], central-shaft type, severely pruned; 2, central-shaft type, moderately pruned; 3, vase type, severely pruned; 4, vase type, moderately pruned.

Plots pruned in winter only: 5, central-shaft type, severely pruned; 6, central-shaft type, moderately pruned; 7, vase type, severely pruned; 8, vase type, moderately pruned; 9, vase type, ringed; 10, lightly pruned type, moderately pruned.



BLOCK A	[1]	1	2	3	[2]	4	5	6	[3]	7	8	9	[4]	10
BLOCK B	8	[1]	9	10	1	[2]	2	3	4	[3]	5	6	7	[4]
BLOCK C	[1]	5	6	7	[2]	8	9	10	[3]	1	2	3	[4]	4
BLOCK D	2	[1]	3	4	5	[2]	6	7	8	[3]	9	10	1	[4]

FIGURE 1.—Plan of orchard used for pruning experiments Numbers in brackets indicate control plots, in which trees were unpruned so far as possible Numbers without brackets indicate plots on which different types of pruning were employed.

On the four control plots in each block the trees were pruned only enough to allow the passage of implements of cultivation, or to remove broken branches.

There were no trees adjacent to the ends of the plots in blocks A and D (fig. 1). Nevertheless the freedom from competition affected each of the 14 plots similarly.

There were trees adjacent to the other two sides, viz, pear trees adjoined plots A10, B[4], C4, and D[4] until February 1924; subsequently orange trees were planted. Adjacent to plots A[1] and B8 there was a row of apple trees, later replaced by citron trees. Adjacent to C[1] and D2 there were apricot trees of the same age and variety as those under experiment.

Field experiments with horticultural crops are admittedly difficult to interpret because there are usually so many unmeasured factors in the equation. After the experimenter has been careful to measure, weigh, analyze, and correlate his data, there are still other, less ponderable, factors which elude his comprehension. The experimenter is fortunate if these more elusive factors affect all his plots to a somewhat similar extent. Then by the laws of chance there will be approximately the same number of plus as minus errors, and they will tend to offset each other, provided a sufficiently large population of trees has been employed in the experiment. In any case the statistical reliability of the data should be tested by means of the probable error of whatever measurements the experimenter used in judging results.

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND INCREMENT IN AREA OF THE CROSS SECTION OF THE TRUNK

VARIABILITY IN WEIGHTS OF WOOD REMOVED

The weight of wood removed will be regarded as the independent variable because it is the factor experimentally varied. The correlations presented in the following pages show the amount of relationship between the above-mentioned variable and others which may be considered as dependent variables.

The weight of wood removed was recorded for all trees immediately after the annual pruning during the dormant season. The weight of the succulent shoots removed in summer from plots 1, 2, 3, and 4 was not recorded because it was not comparable to the weight of the woody shoots removed in winter. For this reason, separate computations were generally made on the four plots which received summer and winter pruning. The records of the size of trees in 1927 were unfortunately lost soon after they were taken.

The amount of wood removed at the winter pruning varied greatly because certain plots were intentionally severely pruned, as stated above. A frequency polygon would show an asymmetrical distribution of the population if classified according to severity of pruning. For example, the average weight of prunings per tree in 1930 was 27.66 pounds; the standard deviation was 25.94 pounds. This great variability is due to the wide range in the individual weights of the prunings, namely, 7 to 157 pounds. It may be considered that the amount of wood removed depended upon the type of pruning employed, the judgment of the man who pruned the trees, the size of the tree, the amount of growth made in the preceding year, and the necessity of forming a framework which could support the weight of new vegetation and fruit. The weight of wood removed is given in table 1.

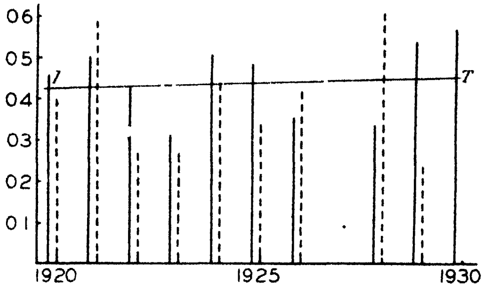


FIGURE 2.—Correlations between weight of wood removed by pruning (*w*) and increase in area of cross section of trunk (*i*) in the succeeding season. Continuous lines represent coefficients of plots 1 to 10, broken lines those of plots 1 to 4, *T T*, line of trend of coefficients of plots 1 to 10.

The growth of the trees was computed from measurements of the circumference of the trunks taken annually. The area of the cross section of the trunk has been found to be a satisfactory index of the size of the tree.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

The coefficients of correlation for weight of prunings (*w*) and increment in growth of the tree trunks (*i*) in the subsequent growing season for individual trees in all 10 plots which were pruned, are shown in figure 2. The broken lines represent the same relationship for individual trees in the four plots which were pruned in the summer as well as in the winter. The coefficients representing the gross correlations for all plots are positive and statistically significant, since the probable errors ranged from ±0.036 to ±0.045.

TABLE 1.—*Average weight of wood removed by the winter pruning (pounds per tree),*[a] *1922 to 1931*

Plot no.	1922	1923	1924	1925	1926	1928	1929	1930	1931
TREES PRUNED SUMMER AND WINTER									
1	59.5±3.64	39.1±2.23	50.4±3.20	67.7±3.67	43.6±3.92	39.0±3.28	45.0±4.78	11.1±0.87	20.4±2.04
2	62.2±2.56	35.6±1.55	52.1±1.67	79.2±2.68	57.6±2.13	42.0±2.57	39.6±2.40	13.1±1.04	19.5±1.50
3	62.9±1.72	44.1±2.18	49.0±1.46	80.5±2.23	56.6±2.25	36.3±2.27	43.8±2.36	10.8±0.88	20.1±1.12
4	65.5±2.15	39.1±1.63	45.8±1.16	74.2±2.11	51.3±1.42	37.6±2.12	48.8±2.72	11.9±0.67	19.9±1.31
TREES PRUNED IN WINTER									
5	71.6±2.76	49.9±2.32	66.0±2.36	80.5±2.41	80.2±2.74	60.6±3.65	84.4±4.48	37.8±2.50	59.8±4.67
6	72.7±2.31	45.1±1.85	68.6±2.49	83.5±2.61	76.0±2.45	65.0±4.02	87.0±4.49	38.8±3.92	53.5±2.67
7	78.3±2.35	62.6±3.46	79.3±2.88	84.1±3.17	83.2±2.55	68.7±3.57	89.6±5.04	42.2±3.92	65.7±4.09
8	80.4±3.00	54.2±3.51	86.9±4.14	110.2±4.68	91.1±3.18	83.8±6.13	123.7±9.02	52.2±5.27	78.0±5.41
TREES GIRDLED									
9	79.6±3.56	40.7±2.72	71.7±3.45	84.4±3.03	78.8±3.22	54.0±3.03	75.5±5.74	29.3±1.91	54.8±3.64
TREES NOT HEADED BACK, MODERATELY PRUNED									
10	41.9±2.52	53.5±4.28	91.4±3.74	64.5±2.02	36.1±2.06	20.9±1.73	35.1±3.04	18.5±1.45	32.1±3.00
CONTROL TREES									
A[1], B[2], C[3], D[4]	57.9±2.98	9.5±1.60	24.2±2.19	37.5±2.98	15.9±2.13	32.6±3.18	10.3±0.87	4.9±0.66	20.8±1.83

[a] The records for 1927 were lost soon after they were taken.

The trend of the coefficients of correlation is shown in figure 2 by the line *T T*. Its equation, determined by the method of least squares, is

$$y = 0.421 + 0.003x$$

The root-mean-square deviation of observed from calculated values is 18 percent of the mean and may be considered as satisfactory, considering the number of observations available. The trend indicates that there would be no material change in the coefficients of correlation, at least for some time.

The nature of the correlation may be illustrated by the results for 1925. Figure 3 shows the regression line of growth of tree trunks on weight of wood removed for 1925. The distribution of the means of y for each class of x is essentially linear and passes through the point *P* which is at the intersection of the lines representing the means of *X* and *Y*. The equation to the line as drawn is

$$y = 11.87 + 0.237x$$

This speaks strongly for the linearity of the regression and the validity of using *r*, the coefficient of gross correlation, as a measure of the association of the two variables

So far as these computations go they indicate that the pruning of these trees was favorable to their growth and that the greater growth followed the more severe pruning

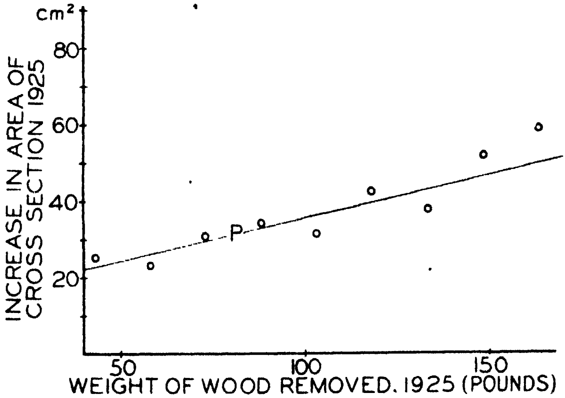


FIGURE 3.—Regression line for means of y for each class of x for 1925, calculated from the equation $y = 11.87 + 0.237x$. Circles represent observed values of y.

The broken lines in figure 2, which represent the coefficients of correlation for plots 1 to 4, which were pruned in the summer as well as in the winter, agree fairly well with the lines for the other set of plots except for the last 3 years. The coefficients of gross correlation show that there was a marked tendency to greater trunk growth on those trees from which more wood was removed. The natural inference that growth tended to increase with severity of pruning is not established, however, until the variation in tree size is taken into account, because the amount of wood removed depends on both severity of pruning and size of tree. There might be a high correlation between wood removed and following growth where only one uniform type of pruning was employed, provided there were great variability in size of tree and in rate of growth with uniform pruning, because of accidental factors such as soil variability. The weight of wood removed from a large tree was generally greater than the weight of wood removed from a smaller tree, even when both were pruned with the same relative severity.

The coefficients of correlation should therefore take into account the size of the trees at the time they were pruned. If we obtain the coefficients of partial correlation between weight of prunings, increment in the area of the cross section of the trunk and that actual area of the cross section at the time of pruning the tree, we may obtain the values of $r_{wi \cdot a}$, the coefficients of correlation between weight of wood removed and increase in the area of the cross section of the trunk; assuming that the trees when pruned all had the same area of cross section (table 2). The coefficients of partial correlation are small in comparison with their probable errors and are therefore without statistical significance. We cannot conclude from them that the amount of wood removed had an important influence upon the growth of the trunks.

Table 2.—*Partial correlation coefficients, $r_{wi \cdot a}$, for the correlations between weight of wood removed by pruning and increments in area of cross section, assuming that all trees were equal in size*

Year	$r_{wi \cdot a}$	Year	$r_{wi \cdot a}$	Year	$r_{wi \cdot a}$
1920	0.031±0.048	1924	0.017±0.048	1928	0.018±0.048
1921	.013± .048	1925	.021± .048	1929	.032± .048
1922	.030± .048	1926	.009± .048	1930	.035± .048
1923	−.004± .048				

AVERAGE SIZE OF TREES EXPRESSED AS A PERCENTAGE OF THE ADJACENT CONTROL PLOTS

We may proceed to investigate another aspect of the problem, namely, "How do the trees in these plots compare in size with the nearest unpruned controls?" This is not a difficult comparison to make because every fourth plot was a control.

If C_1 and C_2 were the average sizes of the trees in the two control plots, then the theoretical sizes for the intervening plots are—

$$p_1 = \frac{3C_1 + C_2}{4}$$

$$p_2 = \frac{2C_1 + 2C_2}{4}$$

$$p_3 = \frac{C_1 + 3C_2}{4}$$

The size of the trees in the 10 plots was computed as a percentage of the adjacent controls by these formulas for 1924 and for 1931 (table 3). The former followed a small crop in which the trunk growth was relatively large; the latter followed several large crops of fruit during which time the trees made less growth than formerly.

One can see that the average area of cross section of these trees became more uniform as the trees grew older. While there were 4 plots in 1924 in which the sizes were distinctly smaller than in the adjacent control plots in 1931, 3 of these 4 plots contained trees whose deviations were ± 5 percent or less, leaving only 1 plot with a deviation greater than 10 percent from the computed size.

TABLE 3.—*Area of cross section of trees in various plots expressed as a percentage of the nearest unpruned control plots*

Time of pruning and plot no.	Year 1924	Year 1931
Pruned summer and winter		
1	85	85
2	95	90
3	92	96
4	100	98
Pruned only in winter		
5	87	95
6	86	96
7	87	98
8	104	108
9	93	98
10	98	107

The reader will likely be impressed (1) by the fact that the average sizes in 8 of the 10 plots, in each year for which data are given, are less than 100 and (2) that most of the deviations are not very great, especially in 1931. The direction of the deviations is, therefore, toward the minus rather than toward the plus side. Have these deviations any significance? If we assume that in an unlimited population the deviations would be zero, then the chance of a deviation of as great as 8:2, or greater, is only 1:17[4] (that is, if pruning is really uncorrelated with growth, 8 or more minus differences are expected only about once in 17 times).

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND YIELD OF FRUIT

The biologist with his interest in the functions of living things, as well as the horticulturist with his interest in the production of orchard fruits, is deeply concerned with this problem. Biologists have investigated the relations of vegetative and reproductive activities of many organisms, the effect of checking one of these activities, and the question of an equilibrium between them. Horticulturists have investigated the question of pruning in relation to the quality of fruit, periodic bearing, the effect of pruning at certain stages of growth, the effect of reducing the number of growing points, and other factors. Few of these problems have been definitely solved by experiments with fruit trees; perhaps some of them never will be. Data which can be analyzed quantitatively are not very abundant in horticultural literature in spite of the importance of the question and of the large number of experiments which have been made.

FREQUENCY DISTRIBUTIONS OF YIELDS

As a first step in studying the yield of fruit the homogeneity of the population was investigated, yields being used as a criterion. The results were assembled in classes differing by 40 pounds each and the frequencies plotted as shown in figure 4.

Most of the yields fell between 100 and 250 pounds. The highest yields were in the 400-pound class, and the lowest in the 40-pound class. The curve is asymmetrical because there were more high-yielding than low-yielding trees. A certain amount of asymmetry is generally found in curves like this. and a comparison of the yields of other years will show that there was generally more asymmetry than in 1922. The greatest numbers of individuals fell into the 160- and 200-pound classes. The actual average yield per tree for 1922 was 194.3 ± 2.8 pounds.

For the purpose of this study the most important result of this arrangement is the evidence that we are dealing with a homogeneous group of trees. If the curve had more than one well-defined hump we should be suspicious that there was more than one biological group in the population, and it would be difficult to study or analyze the data by the simple mathematical methods to be described in subsequent paragraphs.

An inspection of the curves representing the frequency distribution of yields for successive years brings out some significant facts.

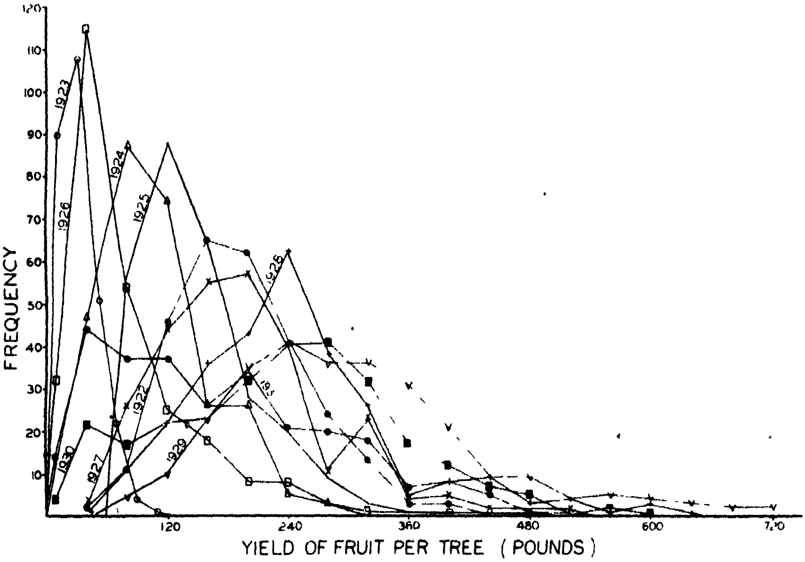


FIGURE 4.—Frequency distributions of yields of all trees, 1922–31

When the yields were large, as in 1929 and 1930, there was a wider "spread" than in years like 1923 and 1926 when yields were small. It seems logical to conclude that small yields were due to the action of factors which had almost similar effects on all trees and that large yields were due to factors which affected the trees diversely. The skewness of the curves to the right indicates that there was a group of persistently higher yielding trees in the orchard. This group (as will be later explained) consisted in large part of the unpruned trees, but some of the plots receiving pruning frequently fell into this class.

The next step in the study is an investigation of the yields of the plots pruned by the 10 systems listed on page 2.

QUARTILE DISTRIBUTION OF YIELDS

The production of 200 individual trees (omitting the records of the unpruned trees in the control plots), the average of their production for the 10 years, 1922 to 1931 inclusive, is shown in figure 5. The trees were classified on the basis of the quartile.[4] The 50 trees producing the least fruit were put into the first quartile, and so on. The actual limits are given in the legend of figure 5. This is an excellent means of expressing the actual production of the population under study and also of observing effects of soil conditions.

The right half of the orchard was less productive than the left half. There was a group of 12 trees in block B all of which were in the first quartile, largely because of die back of the tops, for which no definite cause was found. Another group of low-yielding trees in blocks C and D stood on shallow soil. There is a striking place effect in the marginal trees due to freedom from competition of adjoining apricot trees. Three fourths of the trees in the outside rows were in the fourth quartile. This high production cannot be considered a result of pruning treatment, since the rest of a plot was frequently in a lower quartile. The diagram also enables one to place the various plots with respect to each other. The advantage of the Latin-square method of distributing the subplots is apparent. There is no difficulty in seeing that the yields of the trees in plot 1 were relatively low, while those of plot 8, or plot 10, were relatively high, but a more precise measure can be obtained easily by a simple calculation. There are four quartiles. If the yields of trees were distributed by chance there would be an equal number of trees from each of the quartiles in each plot and the mean quartile value of a plot would be –

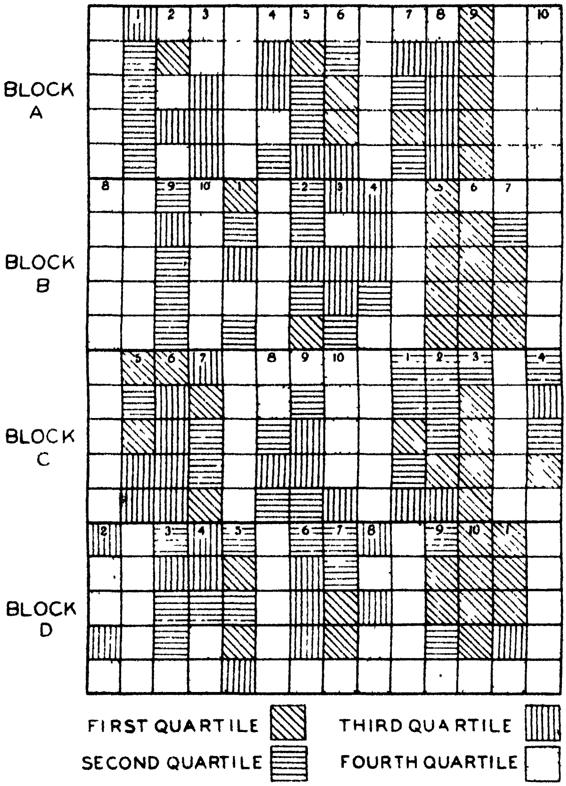


FIGURE 5. Quartile distribution of average yields of individual trees, exclusive of unpruned trees in control plots. Each quartile contained 50 trees. The ranges were: *First* quartile, 75 to 132 pounds, second quartile, 133 to 148 pounds, third quartile, 149 to 172 pounds, fourth quartile, 173 to 273 pounds.

$$n\frac{(1+2+3+4)}{4n}=2.5$$

---

[4] In using the term "quartile" to designate a portion of the population, the author is aware of some danger of confusion of terms. Strictly speaking, a quartile is a point so situated on the base of a frequency polygon that one fourth of the individuals lie on one side and three fourths on the other. In this paper as in the preceding one (*4*),[5] the term quartile will be used to designate one fourth of the population.

[5] Reference is made by number (italic) to Literature Cited, p. 29.

A mean quartile value of less than this indicates that the plot contained more low-yielding trees than would have fallen there on the basis of a purely random distribution; if the mean quartile value is greater than 2.5, the plot contains more high-yielding trees than one would find by chance. The greater the difference, the greater the extraneous influence which determined the mean quartile value. This method affords a ready means of judging the effect of various types of pruning on yields in which the yield of each tree finds expression.

The standard deviation expresses the "scatter" of the individual values around the mean. The standard deviation of the numbers 1, 2, 3, 4 is—

$$\sigma = \sqrt{\frac{4^2-1}{12}} = 1.12$$

That means that if there were an equal number of trees from each quartile in each plot, the standard deviation would equal 1.12. If the observed standard deviation of a plot is less than this, we may justifiably infer that the quartile values cluster more closely to the mean than they would in a purely random distribution. The values under discussion are given in table 4.

TABLE 4.—*Mean quartile values of the yields of apricot trees and standard deviations grouped in plots*

Plot no	M	σ	Plot no	M	σ	Plot no	M	σ
1	2.11	0.85	5	1.80	0.93	8	3.12	0.67
2	2.70	1.05	6	2.35	1.11	9	2.00	.95
3	2.05	1.14	7	1.90	.99	10	3.35	1.19
4	2.90	.85						

There were three plots whose trees had mean quartile values well above 2.5, or, as one might say, contained more than their share of high-yielding trees. We may consider the implications of these results. Plot 8 had the highest mean quartile value and a low standard deviation, indicating that the yields tended to cluster more closely about the mean than they would if the distribution were purely random in character. Figure 5 shows that subplot 8 in block B was on the outside row and that the yield of each tree in that subplot was in the fourth quartile. This position doubtless raised the rating of this plot. However, if these 5 trees had been omitted from the calculations there still would have remained 5 trees of the fourth quartile, 7 trees of the third quartile, and 2 trees of the second quartile. Obviously they rated above the average.

Plot 10 also had a high quartile value, but the standard deviation of the mean is slightly larger than that of a purely random distribution. The results indicate that in spite of variability here is a high-yielding plot, though some of its superiority is due, no doubt, to the marginal position of one of the subplots.

Plot 4 is also conspicuous for a high mean quartile value associated with a rather small standard deviation, yet no subplot was located where it had the benefit of the marginal position.

These three plots had a few characters in common which may deserve comment: (1) Vase-shape trees. All these trees were pruned to maintain open centers, with a consequent production of fruit on short laterals arising from the main limbs. (2) Moderate pruning. The amounts of wood removed were smaller in comparison with the size of the trees than on other plots, especially in the early years of the experiment. (3) Winter pruning was not uniformly better than summer and winter pruning. In fact the yields of plots 4 and 8 are directly comparable in this respect. The results support the arguments in favor of winter pruning, but they are not so wholly conclusive on that point as other results yet to be presented.

Plots 2 and 6, which have mean quartile values and standard deviations differing very little from the theoretical, were pruned alike except that plot 2 was pruned in the summer as well as in the winter.

Plots 1, 3, 5, 7, and 9 have mean quartile values considerably less than the theoretical, indicating that something happened which definitely lowered their fruit production. Plots 1, 3, 5, and 7 were all heavily pruned. The types of pruning on plots 1 and 3 corresponded to those on plots 5 and 7 except that the time varied. Plot 9 falls in this group because the trees were damaged by girdling. The standard deviations of the means are significantly less than the theoretical, indicating that some factor other than chance operated to place them where they are found.

The results of these simple calculations bring out in brief form the relative superiority of the types of pruning employed so far as the total production of fruit was concerned.

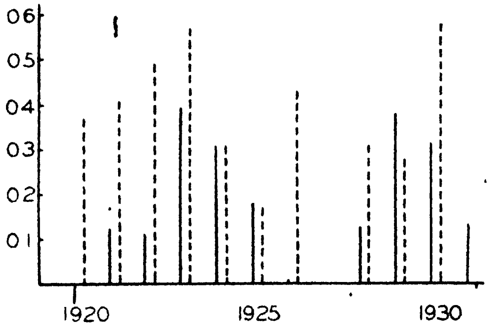


FIGURE 6. Correlations between weight of wood removed by pruning and yield of fruit. Continuous lines represent values of *r* for plots 1 to 10; values less than 0.15 are not significant. Broken lines represent values of *r* for plots 1 to 4.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

We may turn now to the correlations between the amount of wood removed, regardless of the type of pruning, and the yield of fruit.

The coefficients of correlation between w (weight of wood removed at pruning) and y (yield of fruit produced in the following summer) are shown both for plots 1 to 10 and for plots 1 to 4, as vertical lines in figure 6. The magnitude of the correlations is in general surprisingly small and irregular. The values of r_{wy} for plots 1 to 10 were very close to zero in 1920 and 1926. We might expect an absence of correlation in 1920, when, on account of immaturity, the trees produced a small crop which averaged only 18.25 pounds per tree, or in 1926, when the trees produced a crop which averaged only 67.50 pounds per tree. We cannot, however, apply this explanation to the low values of the correlations in 1922 or 1928 when large crops of fruit were produced.

The graphs show that the values of r_{wy} for plots 1 to 10 were positive and significant in 5 of the 11 crops recorded. We may with propriety disregard the small crops of 1920 and 1921 and say that there were positive significant correlations 5 times in 9 years. This is better, but it is not so significant that it can have great value in forecasting the probable results of pruning if yield of fruit is the sole criterion. There are nevertheless some important observations to be made on the coefficients under discussion. It will be noticed that all the significant coefficients are positive. This effectively disposes of any argument that pruning, severe as it was, decreased yield of fruit. Severe pruning means that in some cases the prunings from a single tree weighed as much as 150 pounds. On the other hand, we may safely say that, in 5 of the 9 years, the trees which bore the heaviest crops of fruit were those from which more wood was removed

Figure 6 also contains vertical broken lines which represent graphically the coefficients of correlation, r_{wy}, for the 4 plots which were pruned in midsummer as well as in winter. In 9 of the 11 years the coefficients were positive and more than 3 times their probable errors. Hence the coefficients of these plots indicate a stronger positive correlation between pruning and yield than those of the entire 10 plots.

The question arises, "Did the summer pruning actually affect the physiology of the tree in a way that harmonized the trends in pruning and yield, i.e., heavier pruning and heavier yield, or is this the result of using a more homogeneous population?" The answer involves a knowledge of the variability of the weight of prunings and the yield. The products of the standard deviations are an excellent measure of the dispersion of the values of w and y about their mean values, and are easily obtained from the computations of the various values of r_{wy} for the 11 years. The mean value of $\sigma w \ \sigma y$ for plots 1 to 4 is 766 and for plots 1 to 10 is 1,432. The difference between the 2 products is evidence that the first-named plots of trees were more homogeneous than the last-named plots and accounts for the higher correlations in the case of plots 1 to 4 inclusive. The fact that the summer- and winter-pruned plots produced somewhat less fruit than plots 5 to 10 inclusive suggests that any leveling factor at work tended to reduce the yield of the higher producing trees to average or subaverage amounts.

Further considerations have shown that the inclusion of data from plot 10, the "long-pruned" plot, was responsible for the smaller coefficients of correlation of plots 1 to 10 inclusive. For example, the data was taken for plots 1 to 9 for 1926 (a year in which the correlation was approximately zero). For these plots a value of $r_{wy} = 0.335 \pm 0.045$ was obtained in place of 0.009 ± 0.048. The value of r_{wy} for plot 10 was 0.005 ± 0.132 for the same year. From this it appears that the inclusion of the records of plot 10 had much to do with the lack of correlation indicated above. The discrepancy is due to the fact that the difference between plot 10 and the others was not solely in the amount but in the type of wood removed. The pruning operations on plots 1 to 9 removed the terminal portions of practically all the shoots which had grown in the preceding summer, while on trees of plot 10 they consisted essentially of thinning the shoots without removing the terminal portions. If a branch was shortened by the removal of the terminal portion, it was cut immediately above a lateral branch, avoiding what is ordinarily designated as "heading back."

The influence of the growth of the tree on the values of r_{wy} may be considered next. It needs no lengthy discussion to show that the trees which produced the most new growth had more material removed when pruned, perhaps not relatively, but absolutely. Therefore it is advisable to determine $r_{wy \cdot t}$, the coefficient of partial correlation. The values are shown in figure 7. The coefficients here shown make it more than ever apparent that there is no real correlation between the severity (within limits) of pruning and the yield of fruit. Although there was a small negative correlation between the pruning and yield in 3 of the years, there were 4 years in which the correlations were positive and equally strong. This emphasizes the necessity of experiments extending over a period of several years. If, for example, one had experimented with the trees only when they were young and had seen the negative correlations obtained in 1921 and 1922 he might have concluded that pruning is antagonistic to yield, a relationship which was not seen again until 1926. There were only 4 years in which there were good positive correlations in the plots which were pruned in the summer as well as in the winter.

The coefficients of correlation between weight of wood removed and the yield of the following year were also of doubtful significance. The values of $r_{w_x y_{x+1}}$ were determined for 10 years. Eight of the coefficients were positive; 2 were negative in sign. Their mean value was 0.203. In 4 of the 10 years the correlation coefficient was larger than 0.3 and positive; in 1 year it was less than −0.3. It is evident that there was no such strong correlation as in the case of $r_{t_x y_{x+1}}$, to be discussed later.

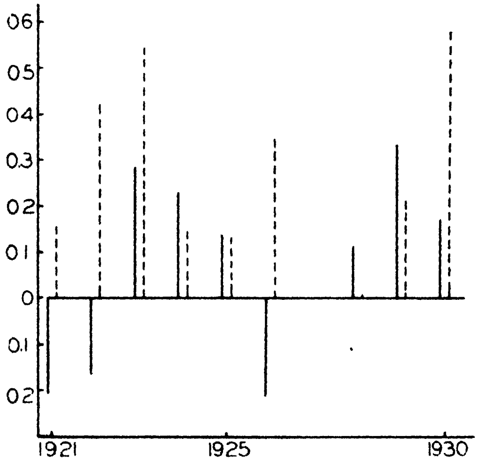


FIGURE 7. Coefficients of partial correlation. Continuous lines represent values of $r_{wy \cdot t}$ for trees in plots 1 to 10, broken lines those for plots 1 to 4.

Summarizing the discussion on this problem, it may be said that there is no evidence that the pruning of these 10 plots of apricot trees in the dormant period had any consistent effect upon the amount of fruit the trees produced in the following summer. If the study is confined to the 4 plots which were pruned in the summer immediately after harvest, as well as in the winter, a positive relationship will be found between the amount of wood removed and the yield of fruit in only 5 of the 9 years for which data are available. The unpruned trees produced more than those pruned by any methods whatsoever, but the quality was so poor that the fruit had no commercial value.

CORRELATION BETWEEN WEIGHT OF WOOD REMOVED AND NUMBER OF FRUITS PER POUND

The correlation between weight of wood removed and number of fruits per pound is a matter of primary importance not only from a biological but from an economic standpoint. Experience has shown that under the climatic and soil conditions in southern California, pruning is an important factor in producing fruit of the required quality.

The size of fruit varied from year to year, but the relative sizes on the 10 plots were in a general way rather constant (table 5). That is to say, the smallest fruits were produced on the unpruned trees and the largest on trees regularly pruned during the winter. Trees pruned in the summer and winter generally produced fruit a little smaller than those pruned only in the winter. There were variations in individual trees which are not readily shown in the averages in table 5, but are reflected in the coefficients of correlation.

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

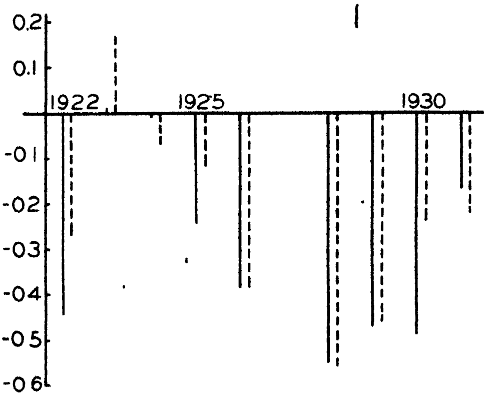


FIGURE 8.—Correlations between weight of wood removed and average number of fruits per pound. Values less than 0.125 are not significant. Continuous lines represent values of r_{wn} for plots 1 to 10, broken lines those for plots 1 to 4.

Correlations between the weight of wood removed during the dormant period and the average number of fruits per pound on each particular tree in the following summer were determined for the plots. After 1928 the data from plots 9 and 10 were omitted because the trees on plot 9 were injured by girdling and the type of pruning employed on plot 10 produced results unlike those on the other plots. The coefficients of correlation r_{wn} are represented graphically in figure 8. In the case of plots 1 to 10, values less than 0.125 are not significant, and in the case of plots 1 to 4, 0.200 is the smallest value having significance, assuming that r must exceed three times the probable error. The coefficients were significant in 7 of the 9 years for which there were records, and all the significant coefficients were negative.

The mean value of r_{wn} was -0.299. The root-mean-square deviation of the nine computed values of r from the mean is 0.200, indicating a rather wide dispersion of the coefficients during the 10-year period. We should predict from these results that the more severe types of pruning would tend to produce fruit of more desirable sizes, especially as the trees grow older, though there might be years in which some other factor (climate or cultural practices) would interfere with the expression of the true relationship. A line of trend is difficult to establish because of the great variability in the first 4 years in the values of r. Fitting a straight line to the data gives the equation $y = -0.140 - 0.029x$, but extrapolation of this line is impossible because of its slope.

TABLE 5.—*Average number of fruits per pound on trees pruned at different seasons and by various methods, 1922–31*

Plot no	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931
TREES PRUNED SUMMER AND WINTER										
1	15.3±0.4	9.3±0.2	10.3±0.2	12.5±0.2	8.4±0.4	12.7±0.2	12.0±0.4	13.9±0.4	14.6±0.4	14.4±0.7
2	17.7± .4	8.3± .2	10.4± .2	12.3± .2	8.5± .4	13.1± .2	12.1± .2	14.7± .4	15.1± .4	13.7± .6
3	15.0± .6	9.0± .2	9.8± .1	13.4± .2	8.0± .2	13.3± .2	11.4± .2	14.5± .4	13.0± .3	16.6± .7
4	18.0± .5	8.9± .2	10.0± .1	14.1± .2	7.7± .3	13.0± .3	10.8± .2	13.7± .4	13.9± .3	16.2± .6
TREES PRUNED IN WINTER										
5	15.0±0.5	8.8±0.2	9.4±0.2	12.5±0.1	8.0±0.4	12.8±0.2	10.9±0.3	13.9±0.4	13.0±0.3	13.4±0.6
6	15.9± .4	8.9± .2	9.5± .2	12.6± .2	8.3± .5	12.3± .2	10.2± .2	12.6± .4	12.7± .3	12.9± .6
7	13.4± .5	8.9± .2	9.1± .2	12.2± .2	8.1± .3	11.9± .2	9.7± .3	13.0± .5	11.6± .2	13.7± .4
8	17.4± .8	7.9± .2	9.7± .1	13.0± .3	7.8± .5	13.3± .2	10.3± .2	13.0± .3	11.9± .3	13.4± .3
TREES GIRDLED										
9	17.6±0.5	9.1±0.2	10.0±0.1	13.1±0.2	8.0±0.2	13.3±0.2	10.6±0.2	15.7±0.4	12.2±0.3	16.1±0.4
TREES NOT HEADED BACK, MODERATELY PRUNED										
10	28.0±0.7	10.9±0.2	10.6±0.1	15.5±0.2	10.1±0.3	19.4±0.3	12.6±0.2	19.6±0.5	14.3±0.2	21.7±1.0
CONTROL TREES										
A [1], B [2], C [3], D [4]	32.7±0.5	12.4±0.2	13.3±0.2	19.3±0.2	10.1±0.3	24.7±0.5	13.4±0.3	21.0±0.7	16.4±0.5	20.3±0.6

The coefficients representing the correlation r_{wn} for the four summer-pruned plots were also significant and negative in 6 of the 9 years. In 5 years the coefficients of the summer-pruned plots were approximately the same in magnitude as those of the nine plots. Two of the three years in which there was no significant correlation between pruning and size of fruit were years in which the crop was so light that the size of fruit was not affected by pruning.

The outcome of these calculations makes it clear that the size of fruit was favorably influenced by the pruning. From time to time there was a year in which there was little if any correlation; yet in the long run there was larger fruit where the trees had been more severely pruned.

The number of fruits per pound was possibly dependent upon the yield as well as on the pruning, although, as previously stated, the severity of the pruning had no very apparent relation to yields. Nevertheless $r_{wn \cdot y}$, the coefficient of partial correlation has been computed, which indicates the degree of correlation which would have existed between the weight of wood removed and the number of fruits per pound if all trees had yielded the same amount of fruit in that particular year (table 6).

TABLE 6. *Coefficients of partial correlation for* $r_{wn \cdot y}$

Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4
1922	−0.561	−0.390	1925	−0.148	−0.282	1929	−0.413	0.460
1923	−.273	−.123	1926	−.387	−.619	1930	.535	.108
1924	−.081	−.142	1928[a]	−.590	−.551	1931	−.338	−.248

[a] The records of plots 9 and 10 were omitted from the calculation

The results presented in table 6 are less variable than the gross coefficients and speak still more strongly, therefore, in favor of pruning as a means of improving the quality of the apricot crop, since only in 1923 and 1924 were these coefficients without significance.

SOME EFFECTS OF REMOVING THE APICAL PORTION OF SHOOTS

The dominant influence of the apical region in controlling the growth of subapical buds has been described in a previous paper (*6*). The results of this work have established the existence of an important physiological action of the apical meristematic region. Horticulturists have long followed the practice of "pinching" or heading back shoots to encourage the formation of laterals where they will be most advantageously placed. Casual observation of the habit of growth of an apricot tree shows that the apical dominance is very strong. The apex of the shoot dominates the growth, not only on the new wood, but on wood produced several years previously. If undisturbed, the apical region has a strong inhibiting action on the development of the fruiting laterals on the older wood. At the same time, the excessive number of spurs on the young wood bear so many fruits that few of them reach a size having a commercial value.

The effects of heading back were clearly shown in comparing the average number of fruits per pound on the plots pruned by different methods. The largest fruits were obtained from plots 5, 6, 7, and 8;

the fruits produced on plot 10 (not headed back) were small and in some years unmarketable. Hence a system of pruning in which the apical region of new wood is continually removed is of value under southern California conditions.

CORRELATION BETWEEN YIELD AND NUMBER OF FRUITS PER POUND

We may next inquire whether the size of the crop had any influence upon the size of the fruit. The results of such an inquiry might be expected to answer the question "Does a heavy yield of fruit mean that the number of fruits per pound will be large?"

COEFFICIENTS OF GROSS AND OF PARTIAL CORRELATION

The values of r_{yn} the coefficient of correlation for these two variables, yield and number of fruit, were determined for each year from 1922 to 1931 inclusive and are represented graphically in figure 9.

The values of the coefficients or correlation for the plots (1 to 10) which were pruned in the winter were largest in 1922, 1925, and 1931, i.e., in years when moderately heavy yields were produced. To be significant the coefficients should have values of 0.15 or greater. In 5 of the 10 years under observation the coefficients are less than three times their probable errors and may therefore be disregarded. The coefficients range from -0.094 ± 0.053 to $+0.530 \pm 0.036$. Their average value is 0.194, which is doubtfully above the threshold of reliability.

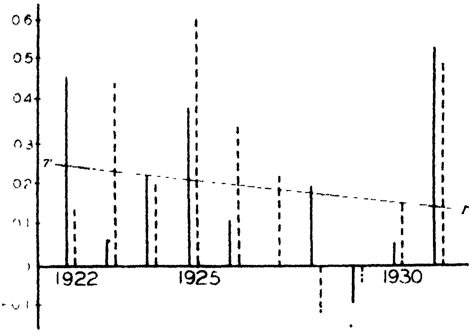


FIGURE 9.—Correlations between yield and number of fruits per pound. Values less than 0.15 are not significant. Continuous lines represent values of r_{yn} for plots 1 to 10, broken lines those for plots 1 to 4; *T T*, line of trend.

The coefficients indicate that in half the years larger crops were correlated with more fruits to the pound, i.e., smaller fruits, but through the 10-year period there is no strong evidence that small crops meant large fruits.

The fruit on these trees was thinned in 1924, 1925, 1927, and 1928. In 3 of the 5 years in which the positive correlations were significant the crops had been thinned. Thinning consisted of removing all but 1 from the clusters of 2 to 6 fruits in late April before the contents of the seeds had lost their gelatinous consistency. This operation is in harmony with good horticultural practice in the locality under consideration. Theoretically the thinning would reduce the value of the coefficient of correlation because trees bearing a large crop would have larger fruits as a result of the thinning. There may have been some reduction of this kind, but there is little weight for such an assumption if one notes the values of the coefficients for 1929 and 1930, when large crops were produced. It was only in 1927 that thinning coincided with a lack of significant correlation.

These coefficients make it obvious that there is no such fixed relationship between yield and size as might have been forecast since only about half the correlations are significant. The large crop of 1929 had a small minus correlation, but other large crops in 1922 and 1928 were positively correlated with fruit size.

The equation to the line of trend, calculated by the method of least squares, is

$$y = 0.259 - 0.012x$$

The line of trend indicates that the correlations would tend to diminish with the increasing age of the trees. That is to say that other factors than yield would probably determine the size of fruit. Among these factors we can recognize (1) competition between the root systems as the trees reached maturity, (2) light conditions, which are often unfavorable to the fruit wood in the interior of large trees in spite of the system of pruning employed, and (3) pruning. It is probable therefore that size of fruit and size of crop will not be definitely correlated more than half the time.

The coefficients of correlation for the plots which received summer and winter pruning are in five of the cases larger than the coefficients for plots 1 to 10, but in a general way they are in harmony with those of the entire 10 plots. The most pronounced disagreements were in 1922, 1927, and 1928.

Since it has been shown that the severity of the pruning (weight of wood removed) affected both the number of fruits per pound, and, to a smaller degree, the yield, it was advisable to determine the coefficients of partial correlation $r_{yn \cdot w}$. These coefficients (table 7) are somewhat larger than those of gross correlation. Six of the nine in the table are large enough to have statistical significance. These figures do not, however, contribute definite support to the general assumption that there is much correlation between yield and size of fruit. This absence of correlation between fruit size and magnitude of yield has also been observed in orange yields (7). The general problem merits further careful study.

TABLE 7. - *Coefficients of partial correlation for $r_{yn \cdot w}$, 1922–31*

Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4	Year	Plots 1 to 10	Plots 1 to 4
1922	0.465	0.322	1925	0.450	0.634	1929	-0.035	0.105
1923	.067	.432	1926	.124	.600	1930	.252	.367
1924	.237	.232	1928	.320	.064	1931	.773	.502

CORRELATION BETWEEN YIELD AND THE GROWTH OF THE TREE

Correlation between yield and the growth of the tree is another problem of biological importance because it bears directly upon the question as to whether the process of fruit production is more or less opposed to vegetative growth. An investigation of the data in hand ought to afford some information on this question. By many investigators the phenomenon of bearing in alternate years in certain trees is generally referred to the competition between fruit production and vegetative growth. Where the supply of material is limited the reproductive processes of the organism usually attract the major portion of the material acquired.

COEFFICIENTS OF GROSS CORRELATION BETWEEN THE GROWTH AND YIELDS OF TREES IN THE SAME YEAR

Figure 10 shows the values of the coefficients of correlation, r_{iy} where i is the increase in the area of the cross section for any year and y is the yield of fruit for the same year. The coefficients are not large enough to have significance except in 1927, indicating that over the period of observations there was no well-marked correlation between yields and the growth of the tree in the corresponding year.

The trend of the coefficients was calculated from the observed values by the method of least squares. The equation to the line of trend is

$$y = 0.204 - 0.0049x$$

The mean observed value of the coefficients is 0.194, but the root-mean-square deviation from the values calculated by the equation to the line of trend is 0.194. Since the observed values are so widely dispersed the line of trend as calculated has little significance.

If the processes of reproduction and wood growth were mutually opposed we should obtain coefficients which were negative in sign and significant in magnitude. But clearly there is no evidence of that condition, nor is there any reliable indication in these data that the two processes are positively correlated. There is, as is well known, an opportunity for the apricot tree to grow for 4 months after the crop of fruit has been harvested (*5*).

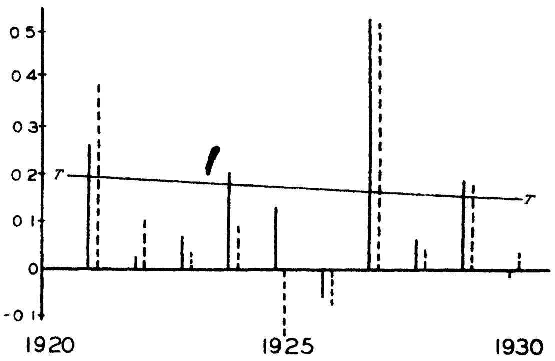


FIGURE 10. Correlations between increment in the area of cross section and the yield of fruit in the same year. Continuous lines represent values of $r_{i_x y_x}$ for plots 1 to 10; broken lines those for plots 1 to 4, *T T*, line of trend.

COEFFICIENTS OF GROSS CORRELATION BETWEEN THE GROWTH AND YIELDS OF TREES IN THE SUCCEEDING YEAR

Another correlation of considerable importance has been determined, namely, the correlation between yields of the trees and their increments of growth in the preceding year. These computations were based on the assumption that the condition of the tree at the end of the growing season (indicated by the increase in the area of the cross section during the whole season) has more influence upon the yield of the tree than the amount of growth made from the blossoming period to time of harvest. Accordingly a series of computations of the values of $r_{i_x y_{x+1}}$ were made, which are represented graphically in figure 11. There is a great difference between the values shown in figures 10 and 11. The coefficients expressing the values of $r_{i_x y_{x+1}}$ are positive and, with one exception, statistically significant. This is good evidence that the yields are more intimately correlated with the growth (measured by area of cross section) of the preceding year than with the growth of the same year.

In both series of computations there is a sag in the value of the coefficients in 1925 and 1926, followed by a conspicuous rise in 1927.

This sag is not readily explained. The average production in 1925, 1926, and 1927 was 148, 73, and 195 pounds of fruit per tree, respectively. The prunings (weight of wood removed) in 1925 and 1926 were 81 and 65 pounds, respectively. The record for 1927 was lost, but it was probably near 60 pounds. The difference, therefore, between the amount of wood removed in 1926 and 1927 was less than between the amounts removed in 1925 and 1926. There is little evidence from these figures that the sag in the values of these coefficients was due to radical differences in the amounts of wood removed by pruning. It is more probable that this sag was related to an infestation of black scale on certain trees in the center of the orchard. The insects appeared in 1924 and increased until exterminated by a period of high temperature which rose to 119° F. on July 17, 1925. The growth increments of the trunks of numerous trees in block B (fig. 1) considerably below the average of the field in 1926, whereas in other years they were generally above the average. It is possible that the retardation of growth by this infestation accounts for the lower correlations noted.

The equation to the line of trend of the coefficients expressing $r_{t_x y_{x+1}}$ is

$$y \quad 0.434 \quad 0.0094x$$

and the mean observed value of the coefficients is 0.377. The deviation of the observed from calculated values is only 0.122, indicating that the calculated values are fairly representative. This indicates that the initially high degree of correlation between growth of the previous year and yield observed in young apricot trees will decline slowly with time. The trees will grow more slowly as they approach maturity, and the yields will be less highly correlated with the annual growth of each antecedent year. As a matter of fact the horticulturist knows that the yields of mature trees are more acutely affected by the incidence of insect infestation or fungous diseases than by other factors which affect directly the physiology of the apricot trees.

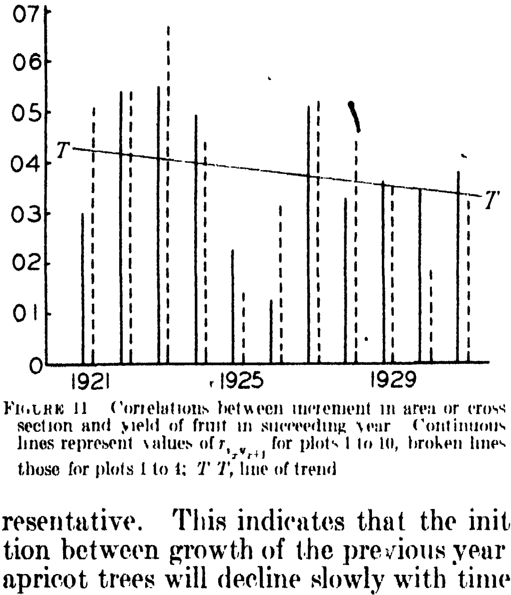


FIGURE 11.—Correlations between increment in area of cross section and yield of fruit in succeeding year. Continuous lines represent values of $r_{t_x y_{x+1}}$ for plots 1 to 10, broken lines those for plots 1 to 4; T T, line of trend

It is unfortunate that so many of the published accounts of horticultural experiments, especially those concerned with pruning, have been conducted on young trees. More reliable information may be had from trees whose root and branch systems fill approximately all the space at their disposal, i.e., when the competition for the components of the soil and for light becomes really acute.

The correlation $r_{t_x y_{x+1}}$ for the trees in plots 1 to 4 (summer-pruned) is so nearly the same that no additional discussion is necessary.

CORRELATION BETWEEN GROWTH OF THE TREE AND NUMBER OF FRUITS PER POUND

COEFFICIENTS OF GROSS CORRELATION

There is a fairly strong correlation between the increment in the area of the cross section and the average number of fruits per pound. The value of r_{in} are shown graphically in figure 12. In 6 of the 9 years for which there are data a fair negative correlation existed. This is as one might expect. The trees that were growing most vigorously were also producing large fruits. In other words, vigorous growth of the vegetative phases of the tree was coupled with vigorous growth of the fruiting organs.

The mean value of r_{in} is 0.272. The trend is represented by a line calculated from the equation,

$$y = -0.260 - 0.0024x$$

The root-mean-square deviation of the observed from the calculated values of y is 0.132, which implies that the line of trend may be accepted with a fair amount of confidence.

On comparing the results of the computations dealing with the relation between the yield and number, and the relation between the yield and growth of the tree, the reader will probably be impressed by the evidence that fruit production in the apricot tree is more nearly comparable to vegetative than to reproductive functions. At all events there is no significant evidence to show that the types of pruning employed restricted the yield of these trees. On the contrary it seems that the growth of the tree was strongly correlated with the yield in the succeeding year, indicating that the two functions go together. This is not surprising when one considers that the yield of fruit depends largely on the thickness of the flesh surrounding the pit, the mesocarp. The mass of the pit is not as variable as that of the fruit, but the thickness of the flesh depends so largely upon the vegetative functions of the tree that it is logical to expect that it should vary with other functions in that category. If it were otherwise one would not expect to see the beneficial results on fruit production obtained from the improvements in the technic of orchard care which are usually obtained in good horticultural practice.

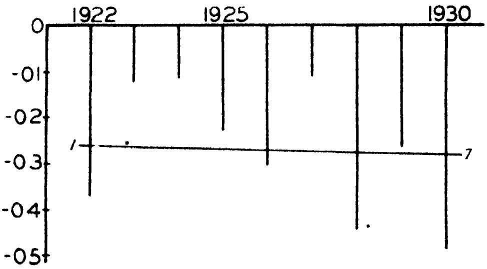


FIGURE 12.—Values of r_{in}, coefficients of correlation between increments in area of cross section and number of fruits per pound. *T T*, line of trend

The small average correlation between the severity of pruning and the yield of fruit, either of the same or of the succeeding year, has been rather fully discussed. It was shown that part of the discrepancy arose from the fact that the system of light pruning applied to plot 10 produced a reaction differing specifically from that obtained from the system of heading-back applied to other trees. This important discovery lends support to the conclusion, reached independently from the other results, that this type of light pruning had little to do with the magnitude of the yields, and that it is not likely to be successful in a locality where apricot trees are prone to produce heavy crops of fruit. The correlation between the severity of the pruning and the number of fruits per pound is more significant and confirms the southern California practice of heading back apricot trees to produce fruit of superior quality.

INTERANNUAL CORRELATIONS

GROWTH OF TREES

One of the problems of paramount interest to the orchardist is the development of his trees. He is concerned not only about the growth of the trees, but about the uniformity of their growth. It is well-known that regularity in growth is generally associated with regularity in fruit production. If the type of pruning or any other cultural operation seriously altered the uniformity of growth of the trees it would sooner or later impair the vitality and productiveness of the trees.

Accordingly some computations on the coefficients of correlation between the size of the trees when measured annually before the growing season started are here presented and discussed. These interannual correlations may be expected to show whether there was any well-defined tendency for the trees of selected plots to grow irregularly and whether the trees which grew most in a given year grew least in succeeding years.

The labor of computing interannual correlations for all plots would be very great; consequently three plots were selected which represent rather distinct types of pruning and the computations confined to them. Plot [1], a control plot, represents the unpruned or nearly unpruned tree. It is of interest because it was well-cultivated, sprayed for pests, and well cared for in all ways except pruning. The trees comprising plot [1] lie on the extreme left side of the orchard, while those in the two other plots were distributed over a larger area (fig. 1). This would vitiate the conclusions if yields or sizes were being compared with those of other plots. However, the yields or sizes of the same trees in different years are being compared, and hence the method of computation is justifiable. Plot 3 was pruned severely. Part of the pruning was done in the summer, but most of the pruning, as already stated, was done during the dormant season. Plot 10 was pruned, but not severely, by thinning branches in contrast to heading-back new growth.

The coefficients of correlation for the area of the cross section of the trees are given in table 8. (The measurements for 1927 unfortunately were lost.) The coefficients are remarkably large, and all are positive. Along the diagonal lines of the table the coefficients indicate that there was almost a 1:1 correlation between the sizes in consecutive years. The coefficients for the correlation of any year with a series of consecutive years show some tendency to form a descending series, but the lowest values in the table are generally larger than those obtained in biometrical work. The coefficients of correlation for plots [1] and 3 between 1930 and preceding years were slightly less than others, though they indicate a strong positive correlation. A satisfactory explanation cannot be given for this depression, nor for the absence of a similar depression in the interannual correlations of plot 10.

TABLE 8.—*Interannual correlations of the area of the cross section of control plot (1), plot 3, and plot 1*

CONTROL PLOT (1)

Year	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0.970±0.038	0.886±0.072	0.707±0.109	0.738±0.101		0.738±0.104	0.665±0.116	0.612±0.122	0.850±0.082
1923		.926± .058	.931± .056	.885± .077		.839± .086	.783± .096	.741± .103	.736± .104
1924			.689± .112	.936± .054		.916± .062	.872± .075	.850± .081	.622± .121
1925				.974± .035		.946± .049	.920± .060	.859± .079	.893± .069
1926						.983± .028	.976± .034	.698± .112	.958± .044
1928							.984± .027	.976± .034	.967± .039
1929								.994± .017	.864± .093
1930									.989± .023

PLOT 3

Year	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0.976±0.033	0.977±0.032	0.948±0.048	0.915±0.061		0.899±0.066	0.868±0.075	0.728±0.103	0.836±0.083
1923		.994± .017	.982± .028	.955± .045		.936± .053	.916± .061	.788± .093	.892± .068
1924			.989± .022	.972± .035		.966± .039	.943± .050	.785± .093	.921± .059
1925				.991± .020		.981± .029	.966± .039	.818± .087	.950± .047
1926						.984± .027	.974± .034	.823± .086	.960± .042
1928							.992± .019	.845± .081	.979± .031
1929								.841± .082	.980± .030
1930									.826± .085

PLOT 10

Year	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0.994±0.017	0.989±0.022	0.914±0.061	0.968±0.038		0.961±0.042	0.943±0.050	0.945±0.049	0.915±0.061
1923		.995± .015	.989± .022	.984± .027		.934± .054	.957± .044	.954± .045	.927± .057
1924			.999± .007	.995± .015		.986± .025	.974± .034	.972± .035	.950± .047
1925				.993± .018		.984± .027	.979± .031	.870± .037	.962± .041
1926						.988± .023	.984± .027	.981± .029	.966± .039
1928							.988± .023	.989± .022	.977± .032
1929								.996± .013	.933± .018
1930									.994± .017

YIELDS

The statistical reliability of the records of fruit-tree yields based on one season's performance is a matter of considerable importance in the study of horticultural problems. When one wishes to lay out experiments he wishes to know whether the yields of an orchard in the year or two immediately preceding the installation of the experiment are significant indices of what the orchard will yield during the term of the contemplated experiment.

Since the labor and expense of obtaining records of the yield of orchard trees are often very great, it is desirable to know whether the record of one or more seasons may reasonably fulfill the requirements of the experimenter. It has been rather implicitly assumed that the average yield for several years is rather more reliable than the record of a single year. The commercial grower is equally interested in knowing whether there is such a close correlation between the crops of successive years that he can estimate with a fair chance of success what any crop yield will be. If it were possible to do this he could effect economies in pruning, spraying, application of irrigation water, fertilizers, and other orchard operations.

The successive yields of the three plots just discussed in the preceding paragraphs were studied in some detail to obtain possible answers to the foregoing questions. The means and coefficients of variability for the 10 years 1922 to 1931 have been assembled in table 9.

TABLE 9.—*The yield and variability of yield of apricot trees through a period of 10 years, 1922–31*

Year	Plot [1] (unpruned)		Plot 3 (severely pruned)		Plot 10 (lightly pruned)	
	Mean yield per tree	Coefficient of variability	Mean yield per tree	Coefficient of variability	Mean yield per tree	Coefficient of variability
	Pounds	*Percent*	*Pounds*	*Percent*	*Pounds*	*Percent*
1922	255.9±5.9	15.1±1.8	141.5±3.7	18.6±2.1	237.5±8.6	24.0±2.7
1923	21.9±1.5	42.9±5.4	45.0±3.7	54.8±7.7	21.8±1.2	35.2±4.2
1924	195.1±6.1	20.3±2.3	70.0±3.4	31.7±3.7	81.5±5.1	41.0±5.7
1925	236.3±11.3	29.6±3.5	128.5±5.0	25.2±2.8	139.5±5.3	25.3±2.9
1926	155.1±12.1	50.2±6.7	67.5±8.6	85.1±14.2	56.5±5.4	63.5±8.4
1927	251.4±12.1	31.1±3.7	178.5±8.3	31.0±3.3	160.5±12.5	51.8±5.5
1928	405.5±19.4	30.7±3.7	226.5±6.5	18.9±2.0	262.0±12.4	31.3±3.3
1929	413.4±16.8	26.3±3.1	288.0±12.1	27.9±3.1	403.0±22.9	37.6±5.2
1930	310.5±22.8	47.4±6.2	237.0±14.6	40.8±10.3	269.0±18.1	44.7±6.7
1931	234.7±17.0	46.7±6.1	190.0±13.6	47.6±7.4	232.0±22.2	63.5±8.4

The large crop of 1922 was followed by a very small crop, but in successive years the mean yields gradually increased until plots [1] and 3 produced about as much in 1927 as they had in 1922. Plot 10 did not equal its 1922 crop until 1928. Each of the three plots produced its maximum yield in 1929.

The coefficients of variability are far from uniform. They are large for 1923 and 1926, when the yields were small, but are also large for 1930 and 1931, when the yields were above the average for the period. It is not surprising that the variability was large when the yields were small because a difference of a few pounds of fruit would then greatly increase the dispersion of the frequency distributions, but the persistence of the variability indicates that there was a factor operating to cause unequal yields in both cases. In fact a distinct trend in each of these plots toward increased variability may readily be recognized during the whole 10-year period. Apparently the inevitable inequalities in soil conditions influenced the yields of the trees more strongly as the extending root systems competed with each other for water and soil components. Plot 3 (severely pruned) was less variable than one or both of the other plots, except in 1923 and 1926. The high variability in 1931 may be attributed partially to the frost injury already mentioned.

Batchelor and Reed (*2*) showed that the variability of tree yields was not significantly decreased by combining the records of two or more years. Parker and Batchelor (*3*) found that the annual gross variability in the yields of orange trees was of approximately the same order, with the possible exception of the first crop. The correlations of yields of individual trees for consecutive years were positive and undoubtedly significant, although the values of the correlation coefficients diminished as time went on.

In contrast with the data on size of trees the yields were more variable at the end of the experiment than the size of trees.

Table 10 contains the coefficients of interannual correlation between the yields of apricot trees in the three plots. There is a somewhat surprising lack of correlation of yields in contrast with the high correlations of area of cross section just discussed.

The diagonal row of coefficients in table 10 shows that there was a significant correlation between yields of trees in control plot [1] in consecutive years only 4 times in 9 years and that each of the 4 significant coefficients had a positive sign. This means that in four cases the trees which produced heavy crops of fruit in a year like 1922 or 1929 tended to produce heavy crops likewise in the year following. However, in 5 out of 9 cases the coefficients do not indicate any significant correlations between the crops of 2 consecutive years.

When we examine the horizontal rows of coefficients we see that the crop of 1922 on control plot [1] was significantly correlated with a subsequent crop 6 out of 9 times. The crop of 1923 was significantly correlated 4 out of 8 times with succeeding crops, and so on. It is very manifest that there was no regular decrease in the values of coefficients similar to what other investigators (*2*, *3*) have found in the growth of annual plants or in the yields of citrus fruits.

The diagonal row of figures in table 10 for plot 3 showed significant correlations between the yields of fruit of the trees in consecutive years only 3 times in 9 years, viz, 1925–26, 1927–28, and 1928–29. The first of these coefficients is negative; the other two are positive. Reference to table 11 shows that the yield in 1925 was moderately large, almost twice as great as that for 1926, with which it had a negative correlation. Evidently the trees in plot 3, which produced more than an average crop in 1925, tended to produce less than an average crop in 1926. The yield in 1927 was fairly large (the largest up to that time), and it was followed by yields increasingly greater in 1928 and 1929. The yield in 1927 was positively correlated with the yield in 1928, and the yield of 1928 was about equally correlated with that of 1929. These relations contrast strongly with those implied in the coefficient of correlation between the 1925 and 1926 yields, indicating that the trees which yielded more than average crops in 1927 and in 1928 tended again to produce more than average crops in the year immediately following.

TABLE 10.—*Interannual correlations of yields for control plot [1], plot 3, and plot 10, 1922-30*

CONTROL PLOT [1]

	1923	1924	1925	1926	1927	1928	1929	1930	1931
1922	0 548±0 129	0 748±0.103	0 495±0.134	0 379±0 143	−0 059±0 154	0 584±0 126	0.761±0.100	0 449±0 138	0 315±0 147
1923		.288± .148	615± .122	.299± .148	.128± .153	.644± .118	.567± .127	.540± .130	−.012± .155
1924			258± .149	.339± .146	.366± .144	.487± .135	.466± .137	.321± .147	.354± .145
1925				.595± .124	−.186± .152	.409± .141	.367± .144	.498± .134	− 200± .152
1926					−.363± .144	.540± .130	.205± .151	.566± .128	−.041± .155
1927						.165± .153	.698± .111	059± .154	.654± .117
1928							.643± .116	872± .149	−.029± .151
1929								473± .133	311± .143
1930									−.371± .140
PLOT 3									
1922	−0 351±0.141	0 259±0.146	0.182±0.148	−0 037±0 151	0 066±0.150	0 500±0.131	−0 048±0 151	0.166±0 149	−0 385±0.139
1923		.390± .139	−.480± .132	− 219± 147	660± 113	.331± .142	.746± .100	.047± .151	.564± .125
1924			−.165± .149	−.400± 150	.554± 125	.154± .149	.409± .138	− 147± .149	.200± .148
1925				− 491± 131	094± 150	100± .139	− 079± .150	− 015± .151	− 448± .135
1926					− 156± 149	074± .150	− 248± .146	380± .139	− 220± .147
1927						.768± .097	.877± .072	.302± .148	.614± .119
1928							.635± .116	.593± .121	217± .147
1929								.226± .146	.750± 100
1930									− 220± 146
PLOT 10									
1922	−0 094±0 150	0 789±0 093	0 514±0 129	−0 055±0 151	0 722±0.104	0 551±0.125	0.660±0 113	0 172±0.149	0 278±0 145
1923		−.157± .149	− 056± .151	.257± 146	225± .147	242± .146	.273± .145	−.177± .149	.291± .144
1924			.388± .139	− 232± .146	698± 108	341± .133	.645± .115	.488± .132	.241± .146
1925				.117± .150	449± 135	658± .111	.237± .147	.416± .137	−.119± .150
1926					− 265± 145	− 027± 151	−.110± .150	− 178± .149	.103± .150
1927						.228± 146	836± 083	.269± .145	458± .134
1928							.105± .150	.075± .151	−.375± .140
1929								.217± .147	.786± .093
1930									−.119± .150

Inspecting the horizontal rows of coefficients in table 10, one finds no distinct trend of the correlations of plot 3, with 1 exception; the yield of 1922 showed no significant correlation with any succeeding crop. The small crop of 1923 shows significant correlations with 4 later crops, 3 of which are positive and 1 negative. A similar state of affairs is pictured by the majority of the remaining coefficients with two exceptions. The yields for 1927 showed a fair positive correlation with 2 of the 5 preceding years and with 3 of the 4 succeeding years. The 1927 yields were positively correlated with the small crops of 1923 and 1924, as well as with the large crops of 1928 and 1929. Thus, out of 9 years there were 5 in which there were indications that the trees yielding more or less than average crops in 1927 maintained their relative positions in the population. In a somewhat similar way the yield in 1928 shows a significant positive correlation with 3 preceding and 2 succeeding crops. The yields of 1928 are correlated with all the larger crops taken from the plot during the period.

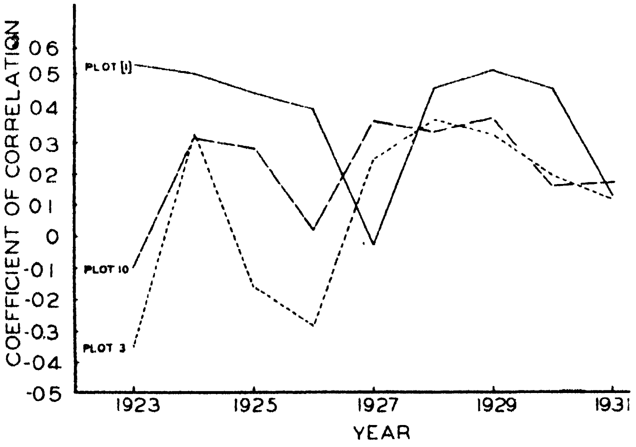


FIGURE 13.—Average interannual correlations of yields of plot [1] (unpruned), plot 3 (severely pruned) and plot 10 (lightly pruned)

The interannual coefficients of correlation between trees in plot 10 may next be examined (table 10). They show somewhat more consistency than those of plot 3, but there is a lack of any well-defined trend. The diagonal row of figures show that there was no definite correlation between the yields of trees in plot 10 in any 2 consecutive years. The coefficients show that the large yield of 1922 was positively correlated with 5 of the 9 succeeding crops, but the small yield of 1923 was not definitely correlated with yields of any of the succeeding years. The yield in 1927 shows somewhat the same degree of relationship to preceding and succeeding years as that of plot 3 showed, viz, a significant correlation with yields in 3 of the 5 preceding and 2 of the 4 succeeding years.

A graphic summary of the interannual correlations of yield is given in figure 13 in the form of 3 graphs representing the average coefficient of correlation for the 3 plots just discussed. The mean for any given year in these graphs is the mean coefficient of correlation between it and the preceding years.

There was no general similarity in the three graphs in 1923, but it should be remembered that each of the points on these graphs for that year is dependent on one coefficient, and has less significance than later values. In each case the values were fairly high in 1924. The average coefficient for the control plot [1] shows a decided minimum in 1927, while the averages for the pruned plots reached minimum values in 1926. The average values in all three cases rose to another maximum in 1929, then declined to 1931. We are not warranted in saying that these small average coefficients are indices to more uniform yields for the years in question. On the contrary the figures in table 9 show that in 1926 and in 1931 the yields were widely dispersed about their means.

The absence of a definite interannual correlation in yields of apricots is rather significant. It indicates that the quantity of fruit produced by a tree is dependent upon factors other than the amount of fruit produced in the preceding year. Whether these determining factors are biological or physical, or a combination of the two, remains to be ascertained. If their nature is biological, such factors as the abundance of pollen-carrying insects, or the prevalence of parasitic insects or of fungi might be investigated. If their nature is physical, it might be temperature, light, water, or that complex assemblage of factors classed as "weather" that was responsible.

The population upon which this study of apricot yields was made was essentially unselected, i.e., heterogeneous, as far as the clon is concerned, although it represented a well-defined type with respect to the species. The variability of the tree yields through the 10-year period was as great as, if not greater than, that commonly found in a random selection of biological material. The variability was likely to be as great in one direction as in another.

In conclusion, the data upon yields of the three plots described show that where annual pruning was practised there was no significant correlation between the yields of successive years and that the correlations between subsequent yields were as large as, or larger than, those of immediately succeeding yields. Significant correlations between yields of unpruned trees were no more numerous than among the plots which were pruned. There is therefore nothing to support the idea that pruning introduced a factor which upset the inherent correlations in yields of consecutive years. It is obviously a matter of chance whether trees which are among the best producers in a given year will produce the best or the poorest crops in the following year. To obtain reliable data on yield it is therefore necessary to collect records for a number of seasons.

SUMMARY

The effects of pruning apricot trees were analyzed by means of the correlation coefficients.

The coefficients of gross correlation show that the amount of wood removed (severity of pruning) and growth of the tree in the succeeding season were positively correlated, but this correlation disappeared when partial correlations involving the size of the tree were made. When the sizes of the tree trunks were computed as percentages of those of the trees on the nearest control plots, the trunks of the trees on 8 plots were found to be smaller and those of 2 larger than the trunks of the trees on the controls. Therefore it seems that trunk growth was somewhat reduced by the heavier types of pruning.

Frequency distributions of the yields gave unimodal asymmetrical curves.

Six of the plots had mean quartile values lower and four of the plots higher than would have been obtained in a purely random distribution of yields. The highest yields for the 10-year period were obtained on plots whose trees were moderately pruned in winter to a vase type. The standard deviations of the means indicate that some factor other than chance determined the average yield of plots. The coefficients of gross and of partial correlation lend little support to the idea that the quantity of wood removed had significant influence upon the yield. The type of pruning was apparently more important than the severity within the limits of the experiment.

The coefficients of correlation show a strong relationship between the amount of wood removed and the average number of fruits per pound. The more severe types of pruning tended to produce fruit of larger size as the trees grew older, provided there was no marked change in environmental conditions.

The results indicate no strong correlation between the size of fruit and the volume of the crop.

The yield of fruit and the growth of the trees in the same season show no very definite correlation, except in 2 years out of 10, but there is a significant and positive correlation between the yield one year and the growth of the tree in the preceding year. Trees pruned in summer and in winter show practically the same correlations as those pruned only in the winter.

There is a fairly strong negative correlation between the growth of the tree and the number of fruits per pound in 6 of the 9 years for which records were kept. The setting of fruit evidently depends on reproductive and vegetative functions of the apricot tree, while the size of fruit probably depends upon much the same factors as do wood and leaf growth.

Interannual correlations of trunk sizes were computed for a control plot, a severely pruned plot, and a lightly pruned plot. All coefficients were positive and very high in value. Apparently size differences were persistent under the conditions of the experiment.

The coefficients of interannual correlations of yields were computed on the three above-mentioned plots. Their values varied greatly from year to year, showing no general correlation. This indicates that the annual quantity of fruit produced by a tree is dependent upon factors other than the amount of fruit produced in the preceding year.

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CORRELATED INHERITANCE OF REACTION TO DISEASES AND OF CERTAIN BOTANICAL CHARACTERS IN TRIANGULAR WHEAT CROSSES[1]

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INTRODUCTION

In order to plan a systematic attack on a plant-breeding problem it is essential to obtain information on the mode of inheritance of certain characters, the reaction of the plant to diseases, and the extent of correlation or linkage, if any, among these various characters. For the purpose of the research presented in this paper triangular crosses were made of Hope, Marquillo, and Supreme, three common spring wheats (*Triticum vulgare* Vill.[3]) to determine (1) the mode of inheritance of reaction to stem rust (*Puccinia graminis* Pers.), bunt (*Tilletia tritici* (Bjerk.) Wint.), and black chaff (*Bacterium translucens undulosum* Smith, Jones, and Reddy), of awn development, and of color of coleoptile; (2) the interrelationship of these characters; and (3) the relation of stomatal behavior to resistance and susceptibility to stem rust. It is recognized, of course, that environment influences many of these characters, making the study of their inheritance very difficult.

REVIEW OF LITERATURE

Considerable work has been done by investigators to determine the inheritance of the two most important characters considered in this paper, namely, reaction to stem rust and reaction to bunt.

REACTION TO STEM RUST

In this paper only those studies having a direct bearing on varietal resistance to stem rust are reviewed.

Melchers and Parker (*32*)[4] found a single-factor difference for rust reaction in a Marquis×Kanred cross. Immunity was dominant when the plants were inoculated at heading time in the greenhouse. Kanred×Marquis crosses have been studied by Aamodt (*1*) and

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[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L.; but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that name.

[4] Reference is made by number (italic) to Literature Cited, p. 55.

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Hayes and Stakman (*26*), who found that one factor may control seedling reaction to several physiologic forms of stem rust. They found also that lines resistant to certain forms of rust in the greenhouse may be susceptible in the field, owing to the presence of other physiologic forms under field conditions or to the difference in reaction of the plants at various stages of growth. Hayes and Aamodt (*24*), working with a Kota × Marquis cross, concluded that the study of F_3 seedlings in the greenhouse was not a satisfactory means of isolating F_3 lines that would exhibit resistance of the Kota type in the field.

Hayes, Stakman, and Aamodt (*27*) crossed Marquis × Iumillo lines, moderately resistant in the mature stage in the field but susceptible in the seedling stage in the greenhouse, with Marquis × Kanred lines, immune in the seedling stage from 11 forms of stem rust. Field resistance was inherited independently of greenhouse reaction to the forms from which Kanred was immune. They concluded that two factors controlled the Marquis × Iumillo type of field resistance in this cross, although minor modifying factors were involved. From these crosses homozygous lines were obtained combining the seedling immunity of the Marquis × Kanred parent with the field resistance of the Marquis × Iumillo parent. There were all combinations of resistance and susceptibility among the hybrids, including homozygous types that contained the factors for resistance of both parents and homozygous types that were susceptible both in the greenhouse and in the field.

Harrington (*20*) reports that there was good agreement between seedling reaction and field reaction of Marquis, Pentad, and Mindum to forms 34 and 21. Tests were made in muslin cages located in the field and the rust forms were checked later in the greenhouse. The greater part of the rust prevalent in 1922 was form 34, and that prevalent in 1923 was form 21. Slight mixtures of other forms also were present.

Aamodt (*2, p. 215*) states: "Immunity or a high type of resistance can be differentiated in the seedling stage in the greenhouse with the expectation that the strains probably also will be resistant in the field." He used the same forms in the field and in the greenhouse.

Goulden, Neatby, and Welsh (*18*), working with a cross of H-44 × Marquis, found no relation between seedling reaction to forms 21 and 36 in the greenhouse and the reaction of the mature plant in the field to a collection of forms. They concluded that different genetic factors controlled resistance in the greenhouse and in the field. Two genetic factors were necessary to explain the seedling reaction to form 36 in the greenhouse. Studies made in the field indicated that resistance was controlled by a single pair of factors.

Clark and Ausemus,[5] in a study of Hope crossed with Marquis and Reliance, found in the mature-plant stage immune, resistant, and susceptible strains breeding true and found also segregating types. The results of the study of the F_2 and F_3 generations were explained on the basis of a 2-factor difference. Phenotypic ratios for the immune, resistant, and susceptible groups were approximately 4:11:1. No susceptible plants or strains were obtained in a Hope × Ceres cross, i.e., a cross between immune and resistant parents. These investigators reported in this cross a single-factor difference, or a 1:3 ratio, for the immune to the segregating and resistant strains.

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[5] CLARK, J. A., and AUSEMUS, E. R. INHERITANCE OF IMMUNITY FROM BLACK STEM RUST, YIELD, AND PROTEIN CONTENT IN HOPE WHEAT CROSSES WITH SUSCEPTIBLE AND RESISTANT VARIETIES. 8 pp. Washington, 1928. [Mimeographed.]

Quisenberry (*39*), in a study of H-44 × Minhardi, found segregation for seedling reaction in the greenhouse of approximately 1 resistant, 2 segregating, and 1 susceptible in a random selection of F_3 lines inoculated with form 60. There was evidence of the influence of minor factors on the expression of rust reaction. The same lines inoculated with form 36 behaved in a similar manner, indicating that the same factors control the reaction of the seedlings in the greenhouse to these two forms of stem rust. However, little relation was found between seedling reaction in the greenhouse to these two forms and the reaction of the mature plants in the field to a collection of forms. He concluded that genetic factors, in addition to those controlling the reaction in the seedling stage, were necessary to explain the reaction in the mature-plant stage.

Goulden, Newton, and Brown (*19*) studied the reaction of seedlings and mature plants of several varieties to 16 physiologic forms of stem rust. They concluded that there were two main groups of varieties: (1) Those in which there was good agreement between seedling and mature-plant reaction, and (2) those in which there was lack of agreement between the seedling and mature-plant reaction.

Goulden (*17*) reported the transference of the resistance of Pentad (durum), *Triticum durum* Desf., to selections of *T. vulgare* in crosses between Pentad and Marquis. Two F_3 lines were obtained in which resistance was homozygous and as high as that of Hope or H-44.

Neatby and Goulden (*34*) found that in a cross of Marquis × H-44 and H-44 × (Marquis × Kanred, B 2-5) field resistance was governed by a single genetic factor, but they concluded that in a Marquis × Hope cross resistance was governed by two complementary factors. In the F_2 and F_3 generations of Marquillo × H-44, it appeared that, in addition to the H-44 factor for resistance, two or more factors were contributed by Marquillo. In the cross Double Cross (Marquis-Iumillo × Marquis-Kanred) × H-44, these investigators assumed that Double Cross carried two complementary factors for resistance. Two factors were concerned in the inheritance of resistance to stem rust in the cross Webster × H-44. Webster contributed a dominant factor for moderate susceptibility, which, when homozygous, was epistatic to the H-44 factor for resistance. They found an extremely low percentage of resistant plants in the F_2 generation in crosses between Marquillo and susceptible varieties. The results indicated that three or more factors were involved in some crosses.

Neatby (*33*) studied the seedling reaction of a Marquis × H-44 cross in the greenhouse and the mature-plant reaction in the field. The mature-plant reaction to a collection of forms was entirely independent of the seedling reaction to a limited number of forms. In a Marquillo × H-44 cross the factors governing seedling reaction in the greenhouse either were expressed in the field reaction or were linked with those for mature-plant reaction. In a cross of Garnet × Double Cross the evidence obtained suggested that there was little or no relation between the factors affecting seedling reaction in the greenhouse and the mature-plant reaction in the field.

Resistance of seedlings in the greenhouse and of mature plants in the field may be due to different causes, each controlled by independent genetic factors. Three types of resistance are known—physiological, morphological, and functional.

Stakman (*40*) has shown that resistance in the seedling stage to physiologic forms of *Puccinia graminis tritici* is due to a physiologic incompatibility between the resistant plants and the invading fungus. The nature of the incompatibility or antagonism between the cereal host and the fungus is not known.

Hursh (*29*) showed that resistance of wheat varieties to *Puccinia graminis* may be due to morphological characters. The pathogene may enter the host plant and develop normally, but the structural characters of the host may prevent extensive development of the rust. The rust mycelium develops almost exclusively in the chlorenchymatous collenchyma of the wheat stem. In morphologically resistant varieties of wheat the collenchyma bundles are small isolated strands, separated by broad bands of sclerenchymatous fibers. This forms a definite mechanical limitation to the spread of the rust mycelium. Morphologically resistant varieties therefore have a large amount of sclerenchyma and a small amount of collenchyma. The relative amount of sclerenchyma increases as the plant grows older.

Hart (*21*, *22*) confirmed and amplified Hursh's conclusions on morphological resistance, and suggested that stomatal behavior of wheat varieties during the early morning hours might be related to stem-rust resistance. She found that the stomata of certain varieties, such as Hope and Webster and certain hybrid lines derived from crosses involving these and certain other varieties, remained closed in the morning later than did those of the susceptible varieties and hybrid lines. This type of resistance was called "functional resistance", and Hart suggests that in some cases mature-plant resistance may be partly functional in nature.

Peterson (*36*) made similar observations on a number of standard varieties and of certain hybrids from two crosses, namely, H-44 × Reward and H-44×Renfrew, and was unable to verify Hart's observations regarding the correlation between stomatal behavior and resistance to rust.

From the standpoint of practical breeding, important forward steps were taken in the production of Marquillo and Hope, two of the varieties considered in this paper. Marquillo was produced by Hayes and his associates (*23*, *25*, *27*) from a cross of Marquis and Iumillo durum, and Hope was produced by McFadden (*31*) from a cross of Marquis and Yaroslav emmer (*Triticum dicoccum* Schrk.) Marquillo has proved moderately to highly resistant under field conditions and Hope is highly resistant in the mature-plant stage to many and perhaps all rust forms of the Mississippi Valley, although susceptible in the seedling stage to some forms.

REACTION TO BUNT

Farrer (*11*, *12*, *13*), of Australia, began in 1901 to develop bunt-resistant varieties of wheat by hybridization, but made no attempt to study the ratios of resistant and susceptible groups. The percentage of bunt in 10 Australian wheats ranged from 12 to 95.5, and Farrer assumed that similar degrees of infection might occur in hybrid generations of crosses of these varieties. He subjected the F_2 and F_3 generations to a heavy bunt attack and selected the bunt-free plants. Hybrid selections were obtained that had 5 to 10 percent of bunt, whereas the susceptible varieties had 80 to 100 percent. In 1905 Farrer made crosses for the specific purpose of getting bunt-resistant varieties. After Farrer's death his assistant, G. L. Sutton, carried on the work and produced two highly resistant varieties, Florence and Genoa.

Pye (*38*) made numerous crosses between Medeah, a highly resistant durum, and *Triticum vulgare* wheats. It was not difficult to obtain resistant hybrids but none of these met the practical requirements of the farmer and miller.

Gaines (*15*) studied the genetics of resistance in the following types of crosses: Resistant × resistant, resistant × susceptible, and susceptible × susceptible. The most susceptible wheats, sown under conditions favoring maximum infection, had an average of 80 percent of bunted heads. Gaines concluded that the freedom from bunt of the remaining 20 percent was due to escapes, for the descendants of these in-crosses showed no evidence of having inherited resistance. Crosses between susceptible varieties produced only susceptible offspring, whereas crosses between resistant varieties produced progeny showing transgressive segregation. Lines were obtained from such crosses that segregated for immunity, resistance, and susceptibility. Gaines studied more than 25 separate crosses and explained the results obtained in each on a multiple-factor basis.

Gaines and Singleton (*16*) assumed that in a cross of Turkey × Marquis two factors controlled resistance, the one carried by Turkey being much more potent than the one carried by Marquis.

Briggs (*5*) made three types of crosses, namely, susceptible × susceptible, resistant × resistant, and resistant × susceptible. The resistant parents were Martin and Hussar, which produced no bunt. The susceptible ones were Hard Federation, Baart, and White Federation, which produced 50 to 95 percent of bunt He concluded that Martin differed from such susceptible varieties as Hard Federation and White Federation in one dominant factor for resistance Later (*7*) he reported that Hussar differed from Hard Federation and Baart by two dominant factors. One of these factors was identical with the factor in Martin but the other was unlike the completely dominant Martin factor, as it permitted about 50 percent of the heterozygous plants to become infected when the Martin dominant factor was absent. Briggs (*6*) later reported that White Odessa had a single dominant factor for resistance similar in its effect to the Martin factor. It was not known whether this factor was identical with the Martin factor. He found (*8*) that Banner Berkeley differed from White Federation in one main factor for resistance to bunt. This factor was identical with the one present in Martin.

Aamodt (*3*) studied the inheritance of resistance to bunt in the F_3 of the following types of crosses of spring-wheat varieties: (1) Susceptible × susceptible, (2) moderately susceptible × moderately susceptible, (3) susceptible × moderately resistant, (4) moderately susceptible × moderately resistant, and (5) moderately resistant × moderately resistant. All degrees of infection were obtained, and in every cross studied a number of F_3 lines transgressed beyond the range shown by either parent. This showed that several genetic factors controlled the reaction to bunt and that numerous recombinations had been produced. Aamodt concluded that multiple factors, the exact nature of which had not been determined, governed the reaction to bunt.

PARENTAL MATERIAL

The three common wheat varieties, Hope, Marquillo, and Supreme, were used as parents in this study. Parental material was obtained from single-head selections from the rod-row trials of each of the varieties grown at the United States Northern Great Plains Field Station, Mandan, N.Dak., in 1927. Table 1 shows the comparable characters of the three varieties.

TABLE 1.—*Comparable characters of 3 wheat varieties*

Character	Hope	Marquillo	Supreme
Reaction to —			
Stem rust	Highly resistant	Moderately resistant	Susceptible
Bunt	Resistant	Semiresistant	Do
Black chaff	Susceptible	Resistant	Resistant
Awnedness	Awned	Awnleted	Awnless
Coleoptile color	Purple	Green	Green

METHODS

Reciprocal crosses were made in 1928. One half of the F_1 plants of each of the three crosses were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D.C.), in the winter of 1928–29. The other half of the F_1 plants and the F_2 plants from the greenhouse were grown at the Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minn., in 1929. In order to obtain as much seed as possible from each plant, the kernels were spaced 6 inches apart in rows 1 foot apart. An F_3 line was grown from each F_2 plant.

The F_3 generation was grown at University Farm, St. Paul, Minn., in 1930. From each F_2 plant duplicate 6-foot rows, containing 25 seeds each, were space-planted for the rust study. An F_2 population was also grown in 1930 from seed obtained from the F_1 plants grown in 1929. Checks of the parents of each cross were grown in every twentieth row.

STEM RUST

Artificial epidemics of stem rust were produced at University Farm, in 1930 and 1931, by increasing physiologic forms of the rust in the greenhouse and hypodermically inoculating at heading time plants of susceptible wheat varieties in the border and alley rows in the field with a water suspension from a mixture of urediospores of all available forms of the rust. Seventeen physiologic forms were used. Separate inoculations with each individual form were also made in the field, but the relative number or prevalence of the different forms on the mature plants at harvest was not determined. Additional infection was induced by similarly inoculating susceptible plants in the heading stage in the greenhouse with all available physiologic forms of rust and transferring them to the field, where they were spaced alternately with inoculated plants at intervals of approximately 20 feet in the alleys and border rows.

Tests of seedling reaction to stem rust, physiologic form 36, were made in the greenhouse. The method of making these tests was similar to that described by Aamodt (*1*) and by Hayes, Stakman, and Aamodt (*27*). The seedlings were inoculated when the first leaf was about 2 inches long.

In these tests duplicate seedings of 20 plants to each 4-inch pot were made. Tests were made of all F_3 lines after taking out the seed necessary for the field tests.

In the study of stem-rust reaction in the field the F_2 plants and the plants of each F_3 line were placed in one of the three classes *R*, *SR*, and *S*. *R* (resistant) includes plants with no infection or only a trace of rust, having a small or narrow linear type of pustule; *SR* (semiresistant) includes plants that appear less resistant than those in *R* and have a type of pustule smaller than that of *S*, the susceptible type; and *S* (susceptible) includes plants having an abundance of rust and well-developed, large pustules that coalesce. The F_3 lines were then grouped according to breeding behavior into the following five classes.

R	Breeding true for *R*
R –	Segregating for *R* and *SR* or breeding true for *SR* type
H	Segregating for *R*, *SR*, and *S*
S –	Segregating for *SR* and *S*
S	Breeding true for *S*

BUNT

In a study of bunt reaction a separate planting was made of F_3 lines infected with the collection of bunt obtained from R. H. Bamberg. No attempt was made to determine the number of physiologic forms present. Twenty-five kernels of wheat were placed in an envelop. After adding a small spoonful of spores, the envelop was shaken until the kernels were completely blackened.

The number of bunted heads was divided by the total number of heads in each line, the results showing the percentage of infection.

BLACK CHAFF

For a black-chaff study an attempt was made to induce an artificial epidemic in the cereal pathology garden, but a better epidemic developed naturally on the material grown in the rust nursery; hence the data on black chaff were obtained from the latter material.

Black-chaff infection was recorded as heavy, medium, and light. The F_3 lines were then grouped according to breeding behavior into the following five classes:

H	Breeding true for heavy infection
H–	Containing plants with heavy and medium infection
S	Segregating for heavy, medium, and light infection
L–	Containing plants with medium and light infection
L	Breeding true for light infection

EXPERIMENTAL RESULTS

REACTION TO STEM RUST

Studies were made in the field on F_2, F_3, and F_4 material and in the greenhouse on F_3 lines. The field studies in 1930 included F_3 families from the entire F_2 population grown the previous year. Some F_4 selections were grown in 1931 to test the accuracy of the F_3 classification.

FIELD STUDIES

Field notes were taken on mature plants, and are referred to herein as mature-plant reaction. The plants in each row were pulled, tied, wrapped, and carefully labeled. The bundles were then shocked under canvas covers to protect the plants from the weather, and the notes were taken the following month

TABLE 2.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of the Hope × Marquillo wheat cross, 1930 and 1931*

Parent or generation		Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
				R	*R*–	*SR*	*H*	*S*–	*S*	Total
Hope	plants	1930		69						69
Marquillo	do			10		38				48
F_2	do			110		214			112	436
Hope	rows			11						11
Marquillo	do				11					11
F_3	lines			33	73		67	22	7	202
Hope	rows	1931	*R*	5						5
Marquillo	do		*R*					1		1
Do	do		*SR*					1		1
F_4	lines		*R*	13	9		13	8		43
Do	do		*SR*		1		9	1	18	35
Do	do		*S*				3	3	21	30

In table 2 are presented data on mature-plant reaction to stem rust in the parent and F_2 plants and in the parent rows and F_3 and F_4 lines of the Hope × Marquillo cross. In considering table 2 it should be noted that the data in the upper three lines pertain to single plants while those below pertain to rows As indicated on page 37, the designation of the plants is somewhat different from that of the rows.

In 1930, all the plants of the Hope parent, grown adjacent to the F_2 plants, were classed as resistant, whereas the plants of Marquillo, similarly grown, were recorded as 10 resistant and 38 semiresistant. There were 436 F_2 plants of the Hope × Marquillo cross, 110 of which were classed as resistant, 214 as semiresistant, and 112 as susceptible, the latter class being unlike the parents (fig. 1). Although in this experiment Hope was more highly resistant than Marquillo, it was not possible to differentiate clearly between the two types of resistance in the F_2 generation. As Marquillo produced both *R* and *SR* plants in the proportion of about 1 to 4, it is probable that 1 out of each 5 plants in the F_2 resistant group may show the Marquillo type of resistance.

All the rows of the Hope parent, grown adjacent to the F_3 lines of the Hope × Marquillo cross, were classified as resistant, whereas all the rows of the Marquillo parent, similarly grown, were placed in the *R*– group. Nine of the Marquillo check rows had a ratio of 1 resistant to 4 semiresistant plants, and two rows had all plants classified as semiresistant.

The 202 F_3 lines were grouped as follows: 33, *R*; 73, *R* –; 67, *H*; 22, *S*– ; and 7, *S*. According to this classification, 33 lines were as resistant as the Hope parent and 106 were within the limits of both parents, whereas 96 exceeded the reaction of both parents.

The results of the studies on the reaction to stem rust in both the F_2 and F_3 generations show that the factor or factors for the mature-plant resistance of the Hope type are not allelomorphic to those of the semiresistant type of Marquillo, as types were obtained that were more susceptible than the semiresistant parent.

A further test of the mode of inheritance of the Hope type of resistance in this cross was made by comparing the number of resistant

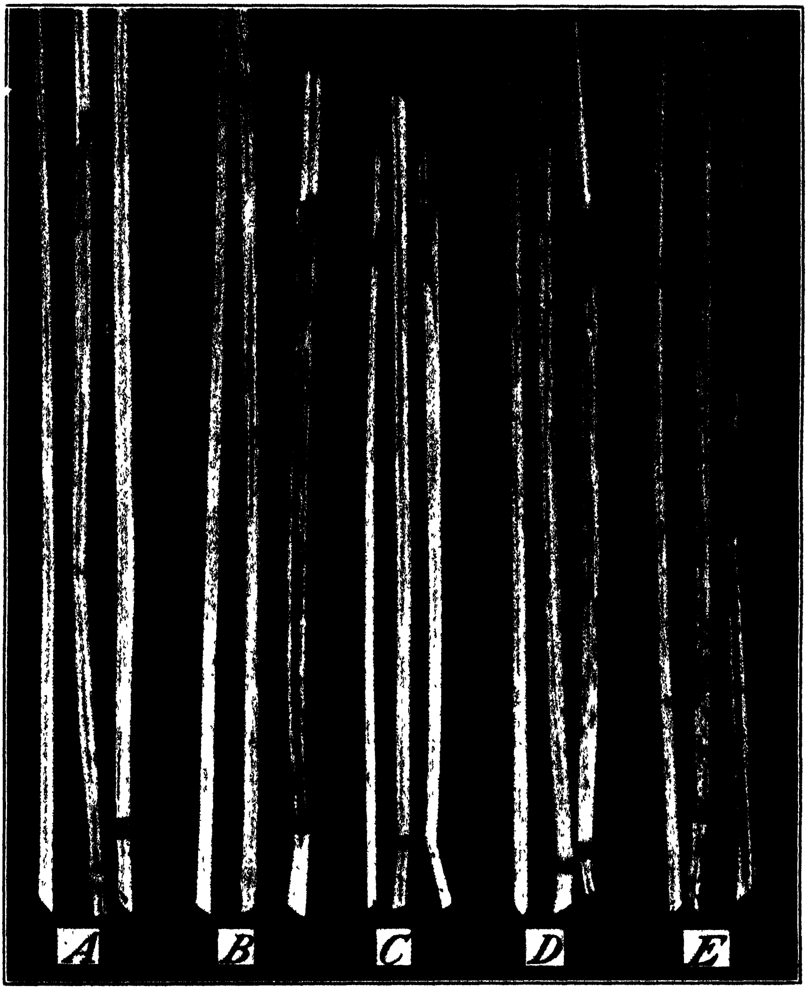

FIGURE 1.—Mature plant reaction to a collection of physiologic forms of stem rust in the field in cross of Hope×Marquillo wheats. Parents: *A*, Hope, resistant; *B*, Marquillo, semiresistant. Segregation of F_2 plants and plants of F_3 lines of Hope×Marquillo cross: *C*, Resistant; *D*, semiresistant; *E*, susceptible.

plants with the combined number of semiresistant and susceptible plants of the individual F_3 *H* segregating lines, according to the method described by Kirk and Immer (*30*). The χ^2 was calculated for each individual F_3 line for a given ratio of resistant to semiresistant and susceptible plants. If the value of χ^2 for a line gave a P value of more than 0.05 (*14, p. 96*), the F_3 line was placed in that particular group.

In each of the individual segregating F_3 lines, the data for the observed number of plants in the resistant group as compared with the observed number in the combined semiresistant and susceptible groups were compared with numbers calculated for the ratios 1:3, 1:15, and 9:7

According to this method of analysis of the 67 F_3 lines segregating for resistant to semiresistant and susceptible plants 50 segregated in a 1:3 ratio, 8 in a 1:15 ratio, and 9 in a 9:7 ratio.

The F_3 lines segregating in a 1:3 ratio would result from a single-factor difference for resistance, and those segregating in a 1:15 ratio would result from duplicate factors for susceptibility. A 9:7 ratio could be obtained from the interaction of complementary factors for resistance. These types of segregation indicate that three or more factors are concerned in the inheritance of rust reaction in this cross. There were too many lines segregating in a 9:7 ratio to be explained by natural crossing. This abnormal ratio may be due, however, to the abnormal chromosomal behavior of Marquillo as found by Powers (*37*), who concluded that Marquillo possesses greater germinal instability than Marquis. Hope is a derivative of a species cross (*31*) and may also exhibit variability in chromosomal behavior.

Other investigators have pointed out the complexity of the problem of studying the reaction to rust as well as to other diseases, as both the host and the pathogene must be considered and both are influenced by environmental conditions.

In 1931 the reaction to stem rust in certain F_3 plants from segregating F_3 rows was studied in their F_4 progeny. All the rows of the Hope parent, grown adjacent to the F_4 progenies, were classed as resistant. One plant of the Marquillo parent classified as resistant and four plants classified as semiresistant in 1930 produced both semiresistant and susceptible plants in 1931. This greater susceptibility of the Marquillo parent in 1931 may have been due to the presence of different physiologic forms in the field or to the influence of environment on the factors governing resistance. The progeny of the F_3 *R* plants of the Hope × Marquillo cross contained approximately 1 homozygous line to 3 segregating for 2 or for all 3 of the rust classes. Of the semiresistant plants 4 lines bred true for the *R* group, but a majority segregated for all classes or bred true for susceptibility. This segregation shows the complexity of the Marquillo type of resistance. The *S* type of plants for the most part bred true.

TABLE 3.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of Hope × Supreme wheat cross, 1930 and 1931*

Parent or generation		Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
				R	*R*-	*SR*	*H*	*S*-	*S*	Total
Hope	plants	1930		37						37
Supreme	do								53	53
F_2	do			228		610			360	1,198
Hope	rows			6						6
Supreme	do								6	6
F_3	lines			6	29		36	25	7	103
Hope	rows	1931	*R*	8						8
Supreme	do		*S*						8	8
F_4	lines		*R*	10	22		7	8		[illegible]
Do	do		*SR*	1	12		2	20	16	51
Do	do		*S*		1			1	29	31

The data in table 3 show the mature-plant reaction to stem rust of F_2 plants and F_3 and F_4 lines of the Hope × Supreme cross, together with the reaction to stem rust of the two parents. These data are graphically presented in figure 2.

When the results obtained in the F_3 generation are compared by the χ^2 method with the calculated number based on a 2-factor difference, $\chi^2 = 11.7524$ and $P = 0.04$. This means that on the basis of random sampling a deviation as great as or greater than that observed could be expected only 4 times out of 100 trials. The agreement between the calculated and the observed ratios is therefore poor in that there were too few lines breeding true for semiresistance and too many lines segregating for all three classes. It is also possible that difficulties in classifying these two groups may partly account for the poor fit obtained. Despite the small number of F_3 lines tested, the results, as a whole, indicate the presence of at least two major genetic factors for rust reaction.

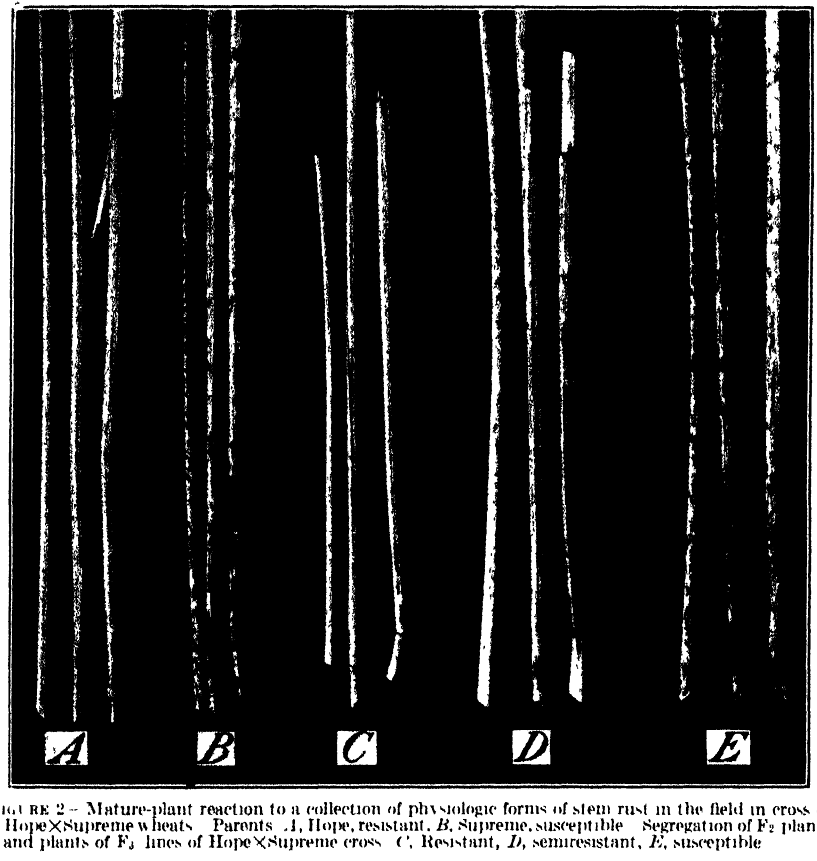

FIGURE 2.—Mature-plant reaction to a collection of physiologic forms of stem rust in the field in cross of Hope×Supreme wheats. Parents: *A*, Hope, resistant; *B*, Supreme, susceptible. Segregation of F_2 plants and plants of F_3 lines of Hope×Supreme cross: *C*, Resistant; *D*, semiresistant; *E*, susceptible.

An analysis of the segregating F_3 *H* lines was made by applying the goodness-of-fit test, as in the cross previously discussed.

Of the 36 F_3 lines segregating for all three classes, 21 lines appeared to segregate for resistant and combined semiresistant and susceptible plants in a ratio of 1:3, and 13 lines in a ratio of 1:15. The *P* values obtained for both types of segregation show a very good fit to a calculated 1:3 and 1:15 ratio, respectively. Two lines that approached a 9:7 ratio, according to this test, may have resulted from natural crossing or mechanical mixtures. The number of lines segregating in the two ratios 1:3 and 1:15 supports the conclusion that at least two genetic factors are involved in the inheritance of stem-rust resistance in this cross.

In 1931 the progeny from *R* F_3 plants from segregating lines included 10 resistant lines and 37 lines segregating for 2 or 3 classes. The progeny of *SR* F_3 plants included 1 resistant line and 50 lines segregating for 2 or 3 classes or susceptible. Plants classified as *S* in the F_3 gave 1 semiresistant line, 1 line segregating for semiresistance and susceptibility, and 29 lines breeding true for susceptibility.

Table 4.—*Mature-plant reaction to stem rust in parent and F_2 plants and in parent rows and F_3 and F_4 lines of the Marquillo×Supreme wheat cross, 1930 and 1931*

Parent or generation	Year	Parent-plant reaction	Number of plants, rows, or lines showing indicated reaction						
			R	*R*−	*SR*	*H*	*S*−	*S*	Total
Marquillo .... plants	1930		7		49				56
Supreme .... do								55	55
F_2 .... do					110			415	525
Marquillo .... rows				7					7
Supreme .... do								7	7
F_3 .... lines				1			60	77	138
Marquillo .... rows	1931	*R*		1					1
Do .... do		*SR*		1					1
Supreme .... do		*S*						2	2
F_4 .... lines		*SR*		2			15	2	19
Do .... do		*S*					2	10	12

The data in table 4 show the mature-plant reaction to stem rust of the F_2 plants and F_3 and F_4 lines of the Marquillo×Supreme cross, together with the reaction to stem rust of the two parents. These data are graphically presented in figure 3.

No plants as resistant as the more resistant plants of the Marquillo parent were obtained in the F_2 generation.

All seven rows of the Marquillo parent were classified as *R*−, each having a few resistant plants. The breeding behavior of the 138 F_3 lines is clearly shown in the table. Although the resistance of the individual plants classified as *SR* was fairly high, it was not equal to that of Marquillo.

Hayes, Stakman, and Aamodt (*27*) concluded from a study of F_3 lines of a Marquillo×(Marquis×Kanred) cross that the resistance of Marquillo was governed by two main factors but that modifying factors also may operate. The results obtained in the experiments herein reported indicate the presence of at least three factors. Seventeen physiologic forms of stem rust were used in the artificial epidemic induced by the writer, while only nine were used by Hayes and Aamodt (*24*). The lower proportion of resistant plants may have been due to the presence of the larger number of physiologic forms or to the nature of the cross. These results and conclusions corroborate those obtained by Neatby and Goulden (*34*) with crosses between Marquillo and susceptible varieties of wheat.

In 1931, two rows each of the Marquillo and Supreme parents were grown. One plant of Marquillo previously classified as *R* and another as *SR* produced similar reactions, both being classified as *R*−,

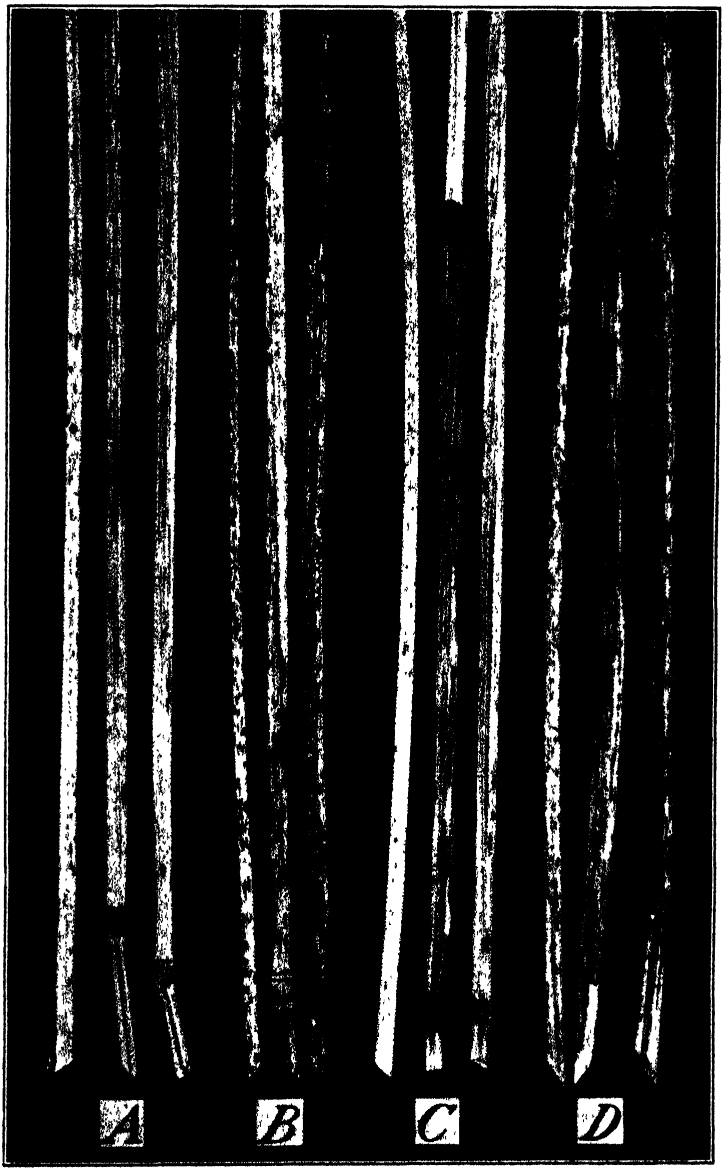

FIGURE 3. Mature-plant reaction to a collection of physiologic forms of stem rust in the field in cross of Marquillo×Supreme wheats. Parents: *A*, Marquillo, semiresistant; *B*, Supreme, susceptible. Segregation of F_2 plants and plants of F_3 lines of Marquillo×Supreme cross: *C*, Semiresistant, *D*, susceptible.

while the two rows of Supreme classed as *S* in 1930 produced only susceptible plants in 1931. The progeny of the F_3 *SR* plants included 2 lines classified as *R* −, 15 as *S* −, and 2 as *S*. The *S* F_3 plants gave 2 lines classified as *S* − and 10 lines that bred true for *S*.

GREENHOUSE STUDIES

The seedling reaction to form 36 in the three parent varieties and in the F_3 hybrids of the Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme crosses was studied in the greenhouse. Hope is highly resistant to form 36 in the seedling stage, and all 296 plants were classed as resistant. All the Marquillo plants were infected, a majority of the pustules being of type 4, indicating susceptibility. Supreme also was susceptible, all plants having the type 4 pustule. All the F_3 lines of the Marquillo×Supreme cross were susceptible.

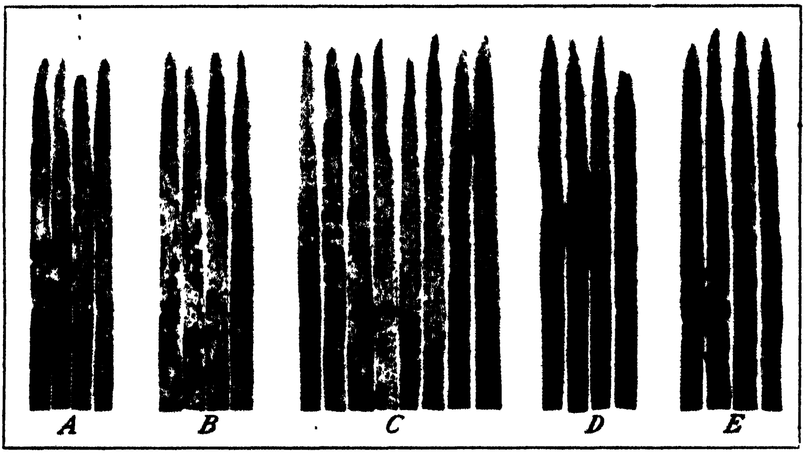

FIGURE 4.—Seedling reaction in the greenhouse to stem rust, form 36, in cross of Hope×Marquillo wheats. *A*, Hope parent, resistant. F_3 of Hope×Marquillo cross: *B*, Resistant; *C*, segregating; *D*, susceptible. *E*, Marquillo parent, susceptible.

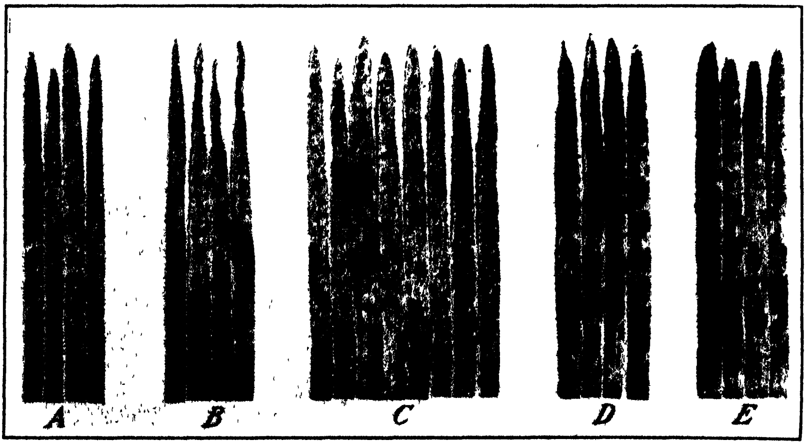

FIGURE 5.—Seedling reaction in the greenhouse to stem rust, form 36, in cross of Hope×Supreme wheats. *A*, Hope parent, resistant. F_3 of Hope×Supreme cross: *B*, Resistant; *C*, segregating, *D*, susceptible, *E*, Supreme parent, susceptible.

The seedling reaction in the two crosses Hope×Marquillo and Hope×Supreme was fairly distinct, so that it was comparatively easy to separate the F_3 lines into three groups—resistant, semiresistant, and susceptible (figs. 4 and 5). Table 5 shows the seedling reaction to form

36 in parent rows and F_3 lines of the three crosses Hope × Marquillo, Hope × Supreme, and Marquillo × Supreme.

Table 5. *Seedling reaction to stem rust, form 36, in parent plants and in F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats, grown in the greenhouse in 1930*

Parent or cross		Number of plants or lines showing indicated reaction			
		Resistant	Segregating	Susceptible[1]	Total
Hope	plants	296			296
Marquillo	do			130	130
Supreme	do			137	137
Hope×Marquillo	lines	46	88	31	165
Hope×Supreme	do	29	46	16	91
Marquillo×Supreme	do			130	130

In the Hope × Marquillo cross the numbers, compared with a calculated 1:2:1 ratio, gave $P=0.18$. In the Hope × Supreme cross the numbers, compared with a calculated 1:2:1 ratio, gave $P=0.16$. These P values and the fact that only one type of segregation was observed in the heterozygous lines indicate that a single genetic factor difference is responsible for the results.

REACTION TO BUNT

Reaction to bunt was studied in the three parent varieties and in the F_3 hybrid lines of the crosses. The F_2 material was not bunted. The parents used in a particular cross were grown, alternately, every twentieth row. The results are presented in table 6.

Table 6. *Distribution of bunted heads in parent rows and in F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats in 1930*

Parent or cross		Number of rows or lines showing indicated percentage of bunt infection							
		0	0.1-3	3.1-6	6.1-9	9.1-12	12.1-15	15.1-18	Total
Hope	rows	16	1						17
Marquillo	do	7	8	3					18
Supreme	do	1	2	3	3	2		2	13
Hope×Marquillo	lines	117	66	12	3				198
Hope×Supreme	do	37	45	12	7	1	1		103
Marquillo×Supreme	do	12	61	34	16	5	3		131

Hope was highly resistant, only 1 row of 17 showing any infection. In the case of the Marquillo parent there was slightly more infection. Supreme was the most susceptible parent, having a greater amount of bunt and a greater range of variability than either Hope or Marquillo. One row of Supreme was bunt free; in others, there was infection in varying amounts up to 18 percent.

The bunt data on the F_3 hybrid lines show segregation approximately covering the range of the respective parents. In the Hope × Marquillo cross 183, or 92.4 percent, of the F_3 lines were within the limits of the Hope parent, and all but 3 were within the limits of both parents. Of the 103 F_3 lines of the Hope × Supreme cross 82, or 79.6 percent, were within the limits of the Hope parent. The bunt reaction in these two crosses was very similar except that there was greater susceptibility in the cross with Supreme, the more susceptible parent. From these crosses with Hope as one of the parents, 154 of 301 F_3 lines grown were bunt free.

Of 131 F_3 lines of the Marquillo × Supreme cross, 12 were bunt free and 107 were within the limits of Marquillo, the more resistant parent. More lines with higher percentages of bunt were obtained in this cross, as might be expected, since neither parent is as resistant as the Hope variety used in the two other crosses.

The results from the three crosses indicate that the greater the susceptibility of one or both of the parents used in a particular cross the greater is the tendency toward susceptibility in the hybrids. Because of the small amount of bunt obtained it is impossible to determine the number of genetic factors involved in the reaction to bunt in the crosses.

REACTION TO BLACK CHAFF

Black chaff affects various parts of the spike of the wheat plant and any or all parts of the culm. The disease sometimes may cause serious losses in the wheat crop. Waldron (*43*) found a decrease in weight of grain and in fertile culms per plant as infection by black chaff increased. In crosses of Hope wheat with other varieties he found an "antagonistic relationship" between resistance to stem rust and resistance to black chaff.

A study of black-chaff reaction is very difficult because little is known of the conditions favoring the development of an epidemic. There usually is a wide range of variability in the reaction of wheat varieties in different parts of a nursery and in different seasons. The data obtained on black-chaff infection in 1930 are presented in table 7.

TABLE 7.—*Reaction to black chaff in parent and F_2 plants and in parent rows and F_3 lines of triangular crosses of Hope, Marquillo, and Supreme wheats in 1930*

Parent or cross and generation	Number of plants, rows, or lines showing indicated reaction						
	H	*H*-	*M*	*S*	*L*	*L*	Total
Hope ...plants	2		18			96	116
Marquillo ...do						101	101
Supreme ...do						86	86
Hope×Marquillo F_2 ...lines	15		83			338	436
Hope×Supreme F_2 ...do	7		99			1,092	1,198
Marquillo×Supreme F_2 ...do						525	525
Hope ...rows				2	8	7	17
Marquillo ...do					1	17	18
Supreme ...do						13	13
Hope×Marquillo F_3 ...lines				11	83	101	198
Hope×Supreme F_3 ...do				3	36	64	103
Marquillo×Supreme F_3 ...do						138	138

The infection of Hope, the susceptible parent, grown adjacent to the F_2 plants and the F_3 lines shows the variability of the epidemic in different parts of the nursery. Segregation appeared to occur in the F_2 generation of the two crosses having Hope as one of the parents, although the infection in most of the plants was classed as light. Definite segregation was less apparent in the F_3 generation, the infection in the larger percentage of the lines being classed as light, as in the Marquillo and Supreme parents.

All plants in the F_2 and F_3 generations of the Marquillo × Supreme cross had a light infection of black chaff and showed no segregation.

AWNEDNESS

The awn development of the F_2 plants and the F_3 lines in the triangular crosses of Hope, Marquillo, and Supreme was studied in 1930. As stated, all the F_2 plants were tested in the F_3 generation in all of the crosses insofar as there was available seed.

HOPE × MARQUILLO

The F_1 hybrids of the Hope × Marquillo cross had awnlets slightly longer than those of the Marquillo parent. The F_2 and F_3 plants grown in 1930 were described in three classes, namely, awned like Hope, strongly awnleted like the F_1, and awnleted like Marquillo. The numbers obtained in the F_2 and F_3 generations were compared with the calculated numbers in a 1 : 2 : 1 ratio by the χ^2 or goodness-of-fit method, giving P values of 0.86 and 0.33, respectively. The results obtained in both the F_2 and F_3 generations, therefore, show a single genetic factor difference for awnedness between the two varieties, Hope and Marquillo. This single-factor difference is similar to that shown by Biffen in 1905 (*4*) and by many recent investigators.

HOPE × SUPREME

In the Hope × Supreme cross and its reciprocal the F_1 plants were apically awnleted or more nearly like the awnless parent. The F_2 plants were grouped in three classes: (1) Awnless like Supreme; (2) a heterozygous group with awns varying in length between the two parent varieties; and (3) awned like the Hope parent. Awn types of the F_3 lines were placed in six groups: (1) Awnless; (2) segregating awnless to awnleted; (3) awnleted; (4) segregating for all types from awnless to awned; (5) segregating awnleted to awned; and (6) awned. The results obtained in the F_2 and F_3 generations showed a very satisfactory fit to a two-factor difference for awnedness when similar numbers were compared by the χ^2 or goodness-of-fit method, P values of 0.12 and 0.07 being obtained.

The assumption of a two major factor difference between crosses of an awned and an awnless wheat is in accord with that of Howard and Howard (*28*), Clark (*9*), Clark, Florell, and Hooker (*10*), and Stewart and Heywood (*41*).

MARQUILLO × SUPREME

The F_1 plants of this cross were apically awnleted and more nearly like the awnless parent. The F_2 plants were placed in two classes: (1) Awnleted like the Marquillo parent, and (2) all plants having shorter awns to awnless, or similar to those of the Supreme parent. It was difficult to separate the awnless and apically awnleted plants because of the variation in a single plant. Therefore, in analyzing the data, these two classes were grouped together. The results in both the F_2 and F_3 generations show a single major factor difference for awnedness between Marquillo and Supreme, when the numbers are compared by the goodness-of-fit method. A P value of 0.40 was obtained in both cases. These results are in accord with those obtained by Stewart and Tingey (*42*).

COLOR OF COLEOPTILE

The first foliage leaf to appear when the young plant emerges from the soil is enclosed in the plumule sheath, or coleoptile. According to Percival (*35*) the coleoptile of wheat may be pale green, colorless, or pink.

The coleoptiles of Hope wheat plants are purple. This color persists, as in H-44 as described by Quisenberry (*39*), after the foliage leaves grow out of the coleoptile; and in some cases the purple tinge shows at the base of the first leaf. Marquillo and Supreme have distinctly pale-green coleoptiles. Data were obtained on the color of the coleoptile of the parents and hybrids in the two crosses of which Hope is one of the parents. Approximately 40 plants were available for the study of each of the hybrid lines, and, in addition, a large number of parent plants were classified. The intensity of coleoptile color was variable and appeared to be influenced by temperature and sunlight at the time of germination and emergence. All the data for an F_3 line were combined to determine the breeding behavior of this character. The plants were placed in two classes only—purplish or green. All Hope plants were classed as purple and those of Marquillo and Supreme as green. A comparison of the numbers obtained for purple, segregating, and green, with the calculated number based on the 1 : 2 : 1 ratio, gives a P value of 0.48. This is a satisfactory fit. Both crosses with Hope as one parent indicate that a single factor is involved in the inheritance of color of coleoptile. In the Marquillo × Supreme cross all plants of the F_3 lines were green.

INDEPENDENCE OF REACTION IN THE CHARACTERS STUDIED

A study was made of the interrelations of the various characters. A measure of the relationship between the distribution of two characters was obtained by calculating χ^2 for independence and by determining the value of P from Fisher's table (*14*). To find the value of P from this table, Fisher's plan in regard to the number of degrees of freedom has been followed. In a fourfold by threefold table in which r = rows and c = columns, $n = (r-1)\ (c-1)$, or six degrees of freedom. The P value is obtained for six degrees of freedom and for the calculated value of χ^2.

The studies of the interrelations of reaction to stem rust (both seedling and mature-plant reaction), bunt, black chaff, awnedness, and coleoptile color are summarized in table 8.

All characters were inherited independently, with the exception of stem rust (field) and black chaff, and stem rust (field) and seedling reaction in the Hope × Marquillo cross. In the reaction of this cross to stem rust there was a tendency to association, probably genetic, of the mature-plant reaction in the field to a collection of physiologic forms with the seedling reaction in the greenhouse to form 36. The data showing this relationship are presented in table 9.

TABLE 8.—*Independent inheritance of the characters in the F_2 and F_3 wheat crosses of Hope, Marquillo, and Supreme*

Cross and generation		Number of plants or lines	Characters compared	n	χ^2	P
Hope×Marquillo F_2	plants	436	Stem rust (field) and black chaff	4	161.09	(a)
Hope×Marquillo F_3	lines	202	do	8	16.64	0.04
Hope×Supreme F_2	plants	1,198	do	4	151.57	(a)
Hope×Supreme F_3	lines	103	do	4	11.25	.03
Hope×Marquillo F_2	plants	436	Stem rust (field) and awnedness	4	2.57	.43
Hope×Marquillo F_3	lines	202	do	8	3.50	.90
Hope×Supreme F_2	plants	1,198	do	1	1.10	.90
Hope×Supreme F_3	lines	103	do	20	12.40	.90
Marquillo×Supreme F_2	plants	525	do	1	.65	.44
Marquillo×Supreme F_3	lines	138	do	3	1.51	.68
Hope×Marquillo F_3	do	198	Stem rust (field) and bunt	12	12.61	.40
Hope×Supreme F_3	do	103	do	9	9.85	.37
Marquillo×Supreme F_3	do	131	do	4	1.58	.81
Hope×Marquillo F_3	do	165	Stem rust (field) and seedling reaction	6	17.22	(a)
Hope×Supreme F_3	do	91	do	8	6.64	.58
Hope×Marquillo F_3	do	165	Stem rust (field) and color of coleoptile	6	6.33	.40
Hope×Supreme F_3	do	91	do	8	6.69	.88
Hope×Marquillo F_3	do	198	Bunt and awnedness	6	2.24	.86
Hope×Supreme F_3	do	103	do	10	14.39	.16
Marquillo×Supreme F_3	do	131	do	8	1.95	.98
Hope×Marquillo F_3	do	164	Bunt and seedling reaction	4	3.79	.44
Hope×Supreme F_3	do	91	do	6	6.01	.43
Hope×Marquillo F_3	do	164	Bunt and color of coleoptile	6	1.53	.96
Hope×Supreme F_3	do	91	do	6	1.82	.93
Hope×Marquillo F_2	plants	436	Black chaff and awnedness	4	2.98	.57
Hope×Marquillo F_3	lines	202	do	4	6.85	.15
Hope×Supreme F_2	plants	1,198	do	4	2.32	.68
Hope×Supreme F_3	lines	103	do	5	7.14	.22
Hope×Marquillo F_3	do	198	Black chaff and bunt	2	.42	.81
Hope×Supreme F_3	do	103	do	3	2.43	.49
Hope×Marquillo F_3	do	165	Black chaff and seedling reaction	1	3.24	.52
Hope×Supreme F_3	do	100	do	2	.33	.62
Hope×Marquillo F_3	do	165	Black chaff and color coleoptile	1	6.49	.16
Hope×Supreme F_3	do	91	do	2	3.87	.16
Hope×Marquillo F_3	do	165	Seedling reaction and awnedness	1	8.08	(a)
Hope×Supreme F_3	do	90	do	10	16.11	.13
Hope×Marquillo F_3	do	165	Color coleoptile and seedling reaction	4	4.51	.35
Hope×Supreme F_3	do	91	do	4	4.36	.37
Hope×Marquillo F_3	do	165	Color of coleoptile and awnedness	4	3.27	.52
Hope×Supreme F_3	do	91	do	10	7.48	.68

[a] Less than 0.01.

TABLE 9.—*Relation between seedling reaction to stem rust, form 36, in the greenhouse and mature-plant reaction to 17 forms in the field in F_3 lines of the Hope×Marquillo wheat cross*

Seedling reaction to form 36 in greenhouse	Number of lines showing indicated mature-plant reaction to 17 forms of stem rust in field				
	R	*R-*	*H*	*S-* and *S*	Total
R	13	14	13	6	46
SR	7	39	33	9	88
S	4	6	14	7	31
Total	24	59	60	22	165

P=less than 0.01.

Of the 24 lines resistant in the field, 13 were resistant to form 36 in the greenhouse, and of the 22 lines susceptible in the field, 6 were resistant to form 36 in the greenhouse. The value of *P* is less than 0.01, showing that the two reactions are not independent. These results are similar to those obtained by Neatby (*33*).

In the two crosses Hope×Marquillo and Hope×Supreme, there appeared to be a tendency to linkage between reaction in the field and to stem rust and to black chaff. This tendency has been observed at University Farm for several years. Table 10 shows the relationship between the two diseases in the field in the F_2 and F_3 generations.

TABLE 10.—*Reaction to stem rust and black chaff in the field in F_2 plants and F_3 lines of Hope×Marquillo and Hope×Supreme wheat crosses*

Reaction to stem rust	Number of plants or lines showing indicated reaction to black chaff			
	HOPE × MARQUILLO, F_2 PLANTS [a]			
	H	*M*	*L*	Total
R	15	57	38	110
SR	0	22	192	214
S	0	4	108	112
Total	15	83	338	436
	HOPE × SUPREME, F_2 PLANTS [a]			
	H and *M*	*L*	*L*	Total
R	66	162		228
SR	39	571		610
S	1	359		360
Total	106	1,092		1,198
	HOPE × MARQUILLO, F_3 LINES [b]			
	H	*L*−	*L*	Total
R	5	18	10	33
R−	2	31	40	73
H	2	28	37	67
S−	1	7	14	22
S	1	1	5	7
Total	11	85	106	202
	HOPE × SUPREME, F_3 LINES [c]			
	H and *L*−	*L*	*L*	Total
R	5	1		6
R−	11	18		29
H	16	20		36
S−	7	18		25
S	0	7		7
Total	39	64		103

[a] P=less than 0.01 [b] P=0.04. [c] P=0.03

There was a tendency to linkage in the crosses in the same direction as in the parents, both F_2 plants and F_3 lines resistant to stem rust being more susceptible to black chaff than the rust-susceptible plants and lines. In spite of this linkage, a considerable number of plants and lines appeared to be resistant both to stem rust and black chaff, and it would seem, therefore, that when Hope is used as one of the parents lines may be obtained from these two crosses that will be resistant to both diseases.

STOMATAL BEHAVIOR IN RESISTANT AND SUSCEPTIBLE WHEAT HYBRIDS

As previously mentioned, Hart (*21*, *22*) has suggested that the "mature plant resistance" of certain wheat varieties and hybrids to stem rust under field conditions may be due to "functional resistance", or the behavior of their stomata, but Peterson (*36*) was unable to verify her observations.

In the present study of stomatal behavior, four F_4 lines of each of the classes—resistant, semiresistant, and susceptible—from the three crosses previously described were selected and grown in 1931, together with the parents. These were space-planted 25 seeds per row in 6-foot rows. The rows were arranged at random for each cross and were examined without knowledge of their identity. The microscopic observations were begun shortly before heading and ended approximately 2 weeks after the heading date of the latest variety or hybrid lines. The first observations were made 30 minutes after sunrise and repeated at 30-minute intervals until the stomata were classed as open Only the stomata on the leaves were studied. They were examined under the low power of a microscope, without the use of the fine adjustment, while the leaves were on the plant. The rows were always examined in the same order, that is, from 1 to 10, 11 to 20, and 21 to 30. It required 9 minutes to examine a series of 10 rows. The stomata of the leaves on the last row examined may have been more widely open that those of the first row because of the extra 9 minutes of sunlight Six leaves of approximately the same age were examined in each row. Only one cross and its parents were examined in a day, and the three crosses were taken in a definite rotation on each succeeding day. The Hope × Marquillo hybrids and parents were examined on the first day, Hope × Supreme on the second, Marquillo × Supreme on the third, etc.

The degrees of stomatal opening were classed as open, half open, narrow slits, and closed. It is more difficult to classify the stomata in the two classes half open and open, as the stomata pass gradually from the closed condition to open; hence the narrow-slit class is the most reliable indication of the comparative degree of opening. A study also was made of the number of stomata in each variety and hybrid line. For this purpose three counts of the number in the microscopic field were made at random on each plot.

Table 11 shows the average date of heading, the average number of stomata, and the average number of 30-minute periods required for a variety or hybrid line to attain a certain degree of stomatal opening. There are three very important factors—light, temperature, and humidity—that cannot be controlled under field conditions. Light intensity appears to be the main factor governing stomatal behavior. Cloudiness affects the rate of opening on any particular day and the time required for the stomata to attain a certain degree of opening on the different days. However, it should be possible to detect stomatal differences in the field if stomatal behavior is the basis of functional resistance or susceptibility, although it would be more desirable to make the study where the various factors could be controlled.

TABLE 11.—*Date of heading, number of stomata, and number of 30-minute periods required for stomata to open in parent and F_4 lines of Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme wheat crosses*

[Data represent averages of observed values]

Variety or cross	Number of 30-minute periods required for indicated degree of stomatal opening			Date of heading (1931)	Number of stomata
	Narrow slits	Half open	Open		
Hope (resistant)	2.6	3.7	4.8	July 10	142
Marquillo (semiresistant)	2.6	3.8	4.9	July 4	131
Supreme (susceptible)	2.6	3.8	4.9	July 7	119
Hope×Marquillo F_4 lines					
Resistant	2.6	3.7	4.7	July 8	147
Do	2.7	3.9	5.0	do	134
Do	2.6	3.9	4.9	July 6	132
Do	2.6	3.9	5.0	July 12	119
Average	2.6	3.9	4.9		
Susceptible	2.6	3.9	5.0	July 8	115
Do	2.7	3.9	5.0	July 6	133
Do	2.5	3.9	4.9	July 10	141
Do	2.6	4.1	5.1	July 4	142
Average	2.6	4.0	5.0		
Hope×Supreme F_4 lines					
Resistant	2.6	3.7	4.9	July 10	164
Do	3.0	3.9	5.1	July 11	174
Do	2.6	3.7	4.7	July 8	181
Do	2.7	3.7	5.0	July 10	136
Average	2.7	3.8	4.9		
Susceptible	2.9	3.9	5.0	July 8	134
Do	2.9	3.9	5.1	July 10	192
Do	2.7	3.7	5.0	do	142
Do	2.6	3.7	4.9	July 5	152
Average	2.8	3.8	5.0		
Marquillo×Supreme F_4 lines					
Semiresistant	2.6	3.6	4.9	July 9	140
Do	2.7	4.0	5.1	July 8	131
Do	2.7	4.0	5.1	do	146
Do	2.6	3.7	4.9	July 9	146
Average	2.7	3.8	5.0		
Susceptible	2.6	3.9	5.0	July 4	138
Do	2.5	3.7	5.0	July 8	171
Do	2.6	3.7	4.9	July 9	130
Do	2.6	3.7	4.9	July 6	161
Average	2.6	3.8	5.0		

The data do not indicate any relation between stomatal behavior and rust reaction in any of the three parents or crosses. Since the resistant lines had no rust, the semiresistant lines a little rust, and the susceptible lines much rust, the differences in stomatal behavior, if any, should also have been consistent and distinct.

Climatic conditions were very abnormal during the period of this study. Temperatures were extremely high and moisture was deficient. As the plants were shorter than they normally are, the flag leaves caught the first rays of the morning sun. This may account in part for the regular opening of the stomata on the different varieties and hybrid lines.

One of the difficulties encountered in this study was the differences in the stage of development due to different dates of heading, as indicated in table 11. Hope headed on July 10, Marquillo on July 4, and the Hope×Marquillo lines varied from July 4 to July 12, extending 2 days past the date of the late-heading parent. Supreme headed on July 7, while the hybrid lines with Hope as the other parent headed from July 5 to 11. The heading dates of the Marquillo×Supreme hybrids ranged from July 4 to 9, most of them heading later than either parent. However, there does not appear to be any correlation between the date of heading and the time of opening of the stomata; it therefore appears that the differences in heading dates do not invalidate the conclusions.

The average number of stomata in the microscopic field was 142 for Hope, 131 for Marquillo, and 149 for Supreme. In the Hope×Marquillo hybrids the average number of stomata ranged from 115 to 149; in the Hope×Supreme hybrids, from 134 to 192; and in the Marquillo×Supreme hybrids, from 130 to 171. There appears to be no relationship between the average number of stomata in a particular line and its resistance or susceptibility to stem rust. These results are in agreement with those obtained by Hursh (*29*).

SUMMARY

Studies were made to determine the manner of inheritance of reaction to stem rust, bunt, and black chaff and of awnedness and color of coleoptile in triangular crosses of the three spring-wheat varieties Hope, Marquillo, and Supreme. A study was made of the inheritance of each individual character and of the independence of different combinations of characters. The relationship between stomatal behavior and mature-plant reaction to stem rust in the field was also studied on the parents and F_4 lines of the triangular crosses.

Epidemic conditions of stem rust were obtained by increasing individual physiologic forms of the rust in the greenhouse and hypodermically inoculating at heading time the plants of the susceptible wheat varieties in the borders and alleys in the field with a water suspension from a mixture of urediospores of all available forms of the rust. In addition, susceptible varieties of wheat were grown in the greenhouse to the heading stage, inoculated with all the available physiologic forms of stem rust, and then transplanted to the field. Bunt infection was obtained by dusting the grain with chlamydospores before sowing. Black-chaff infection was studied under natural infection conditions in the rust nurseries.

In the Hope×Marquillo cross, inheritance of mature-plant reaction to stem rust appeared to depend on three or more factors. Inheritance of the mature-plant semiresistance of Marquillo appeared to depend on factors that were not allelomorphic to those controlling inheritance of the mature-plant resistance of the Hope type. In the Hope×Supreme cross, inheritance of the mature-plant resistance to stem rust appeared to depend on at least two factors. In the Marquillo×Supreme cross, at least three genetic factors appeared to be concerned in the inheritance of mature-plant resistance and susceptibility to stem rust.

Seedlings of the F_3 lines of the Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme crosses were inoculated in the greenhouse with physiologic form 36 of stem rust. The F_3 lines of Hope×Marquillo and Hope×Supreme segregated approximately in the ratio of 1 resistant to 2 segregating to 1 susceptible. All the F_3 lines of Marquillo×Supreme were susceptible.

It was impossible to determine the number of genetic factors involved in the inheritance of reaction to bunt of the three crosses studied, but the results indicate that the greater the susceptibility of one or both parents the greater the tendency toward susceptibility of the hybrids.

Black-chaff infection was light. In general, segregation occurred in the two crosses having Hope as one of the parents, but no conclusion could be reached concerning the number of factors involved in the inheritance of this character. Segregation did not occur in the Marquillo×Supreme cross.

Hope is awned, whereas Marquillo is awnleted. Segregation of this character indicates that a single genetic factor is involved. In the Hope×Supreme cross, in which the Supreme parent is awnless, segregation indicates that two genetic factors are involved. In the Marquillo×Supreme cross one genetic factor appeared to explain the segregation.

Seedlings of Hope have a purple coleoptile in the greenhouse, whereas those of Marquillo and Supreme have a green coleoptile. Segregation of this character in the two crosses having Hope as one of the parents indicates that a single factor is involved. Marquillo and Supreme have distinctly pale-green coleoptiles. In the Marquillo×Supreme cross all plants of the F_3 lines were green.

Independence of reaction to all combinations of stem rust, bunt, and black chaff, and to awn development, and to coleoptile color was studied by means of the χ^2 test.

The inheritance of the following combinations of characters appeared to be independent: Mature-plant reaction to stem rust in relation to bunt, seedling reaction (except in Hope×Marquillo), awnedness, and color of coleoptile; black chaff in relation to bunt, seedling reaction, awnedness, and color of coleoptile; bunt in relation to seedling reaction, awnedness, and color of coleoptile; awnedness in relation to seedling reaction and color of coleoptile; and seedling reaction in relation to color of coleoptile.

There appeared to be a tendency to linkage or association of mature-plant reaction to stem-rust and seedling reaction in the Hope×Marquillo cross and mature-plant reaction to stem rust and reaction to black chaff in the crosses of Hope with Marquillo and Supreme.

The relationship between stomatal behavior and reaction to stem rust was studied microscopically under field conditions. The tests included resistant, semiresistant, and susceptible hybrids from the three crosses Hope×Marquillo, Hope×Supreme, and Marquillo×Supreme. Only slight differences were noted in the stomatal behavior in 1931 and these did not appear to be correlated with rust reaction. It therefore would appear that the resistance of Hope is not due to stomatal behavior.

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CORRELATED INHERITANCE OF REACTION TO STEM RUST, LEAF RUST, BUNT, AND BLACK CHAFF IN SPRING-WHEAT CROSSES[1]

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INTRODUCTION

For more than 20 years cooperative studies to develop rust-resistant varieties of wheat have been under way between the Bureau of Plant Industry of the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. In recent years the objective has been to breed high-yielding, high-quality varieties of wheat resistant not only to stem rust (*Puccinia graminis* Pers.) but to other important diseases, such as leaf rust (*P. triticina* Eriks.), bunt (*Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn), scab (*Gibberella saubinetii* (Mont.) Sacc.), black chaff (*Bacterium translucens undulosum* Smith, Jones, and Reddy), and root rots, principally those caused by *Helminthosporium sativum* Pam., King, and Bak., and *Fusarium* spp.

Hope and H-44, the varieties used most recently as the parents resistant to stem rust, leaf rust, and bunt, are very susceptible to the bacterial black-chaff disease. Observation and certain experiments indicate that there is some tendency for resistance to stem rust and susceptibility to black chaff to be associated in the segregates of crosses having as one of the parents either Hope or H-44 or a related strain. The experiments reported in this paper were conducted to ascertain the extent of genetic linkage or association of these diseases. It is thought that the results may supplement in a useful way a companion contribution by one of the writers.[3]

Since most of the pertinent literature has been reviewed in the companion paper referred to, it seems unnecessary to present such a review here. An abstract of a paper presented at the Sixth International Congress of Genetics at Ithaca, N.Y., in 1932, gives a brief outline of the studies in Minnesota that have led to the present appreciation of the importance of mature-plant resistance.[4]

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MATERIALS AND METHODS

The experiments reported herein were conducted in 1929 and 1930 at University Farm, St. Paul, Minn. Those for the first season were limited to a cross between H-35 and Marquis and those for the second season to crosses in which H-44 was used as one parent and Kota × Marquis No. II-19-167 or Double Cross No. II-21-28 as the other. H-44 and H-35 were obtained from crosses between emmer and Marquis wheat. They have 21 chromosomes, and so far have been highly resistant to stem rust under field conditions. H-44 has been resistant to leaf rust and also to bunt. Both are very susceptible to black chaff.

Kota×Marquis No. II-19-167 and Double Cross No. II-21-28 were selected as parents on the basis of preliminary yield experiments and milling and baking tests. They appear to excel in agronomic characters and in milling and baking quality. They are moderately resistant to stem rust, highly resistant to black chaff, susceptible to leaf rust, and moderately susceptible to bunt.

The parents and F$_3$ lines were grown in special nurseries, the plants being spaced 3 inches apart in rows 1 foot apart.

The F$_3$ lines were from a random selection of F$_2$ plants. Approximately 10 rows of each parent were grown at regular intervals within each series of F$_3$ lines. One or two rows of each line, each row containing 25 seeds, were sown in blocks 6 by 132 feet, thus allowing 132 rows to a block. The blocks were surrounded by rows of susceptible varieties sown crosswise through the alleys and in the border rows. A rust epidemic was induced by dusting urediospores on plants in the heading stage in the greenhouse and then transplanting these inoculated plants to the border and alley rows in the rust nursery. In addition, a water suspension of urediospores of the available forms of stem rust was injected hypodermically into the upper leaves of plants in the heading stage in the border and alley rows. This method has been very satisfactory.

Bunt was studied in a separate nursery; black chaff was studied in the rust nursery. For the bunt studies the wheat was inoculated by dusting with bunt spores just before sowing. Black chaff developed from natural infection.

RUST

In order to compare the reaction of the various F$_3$ lines to stem rust and leaf rust the plants of each F$_3$ line were placed in one of three classes, designated *R*, *SR*, and *S*. *R* includes plants with no infection or only a trace of rust of the type of pustule commonly associated with resistance; *SR*, or semiresistant, includes plants that appeared rather resistant, the type of pustule being smaller than in the susceptible group; and *S* includes susceptible plants on which there were large confluent pustules and severe infection. Each F$_3$ line was classified on the basis of reaction of all F$_3$ plants. The lines were then grouped according to breeding behavior. The groups for reaction to leaf rust and stem rust were as follows:

R — Breeding true for *R*.
R— — Containing *R* and *SR* plants, or breeding true for *SR* type.
H — Segregating for *R*, *SR*, and *S*.
H— — Segregating for *SR* and *S*.
S — Breeding true for *S*.

BUNT

The number of bunted spikes was divided by the total number of spikes in each line, the result showing the percentage of infection. The counts were made separately for each parental and F_3 line.

BLACK CHAFF

Notes on black chaff were taken on individual plants and the degree of infection was indicated as heavy, medium, or light. The lines were then grouped into 7 classes, as follows: Class 1, very heavily infected and apparently homozygous for susceptibility; classes 2 to 6, intermediate degrees of reaction; classes 3, 4, and 5, apparently segregating and containing both highly susceptible and apparently very resistant plants; class 7, highly resistant and showing only slight traces of black chaff.

EXPERIMENTAL RESULTS

The results of the experiments on reaction to individual diseases are presented first and are followed by a study of independence in reaction to the various combinations of diseases.

REACTION TO STEM RUST

The reaction to stem rust is summarized in table 1. Comparisons are shown between the reaction of the F_3 lines from the crosses of H-44 with Kota × Marquis No. II-19-167 and with Double Cross No. II-21-28 and the reaction of the parent rows, in 1930, and the reaction of the progeny of F_3 plants in the F_4 generation, in 1931. The purpose of the F_4 test was to determine the accuracy of the F_3 classification.

TABLE 1. *Stem-rust reaction in parent rows and F_3 and F_4 lines of three wheat crosses grown at University Farm, St. Paul, Minn., 1929, 1930, and 1931*

Parent or generation		Year	Parent-plant reaction	Number of rows or lines showing indicated reaction					Total
				R	*R*	*H*	*H-*	*S*	
H-44	rows	1930		8	2				10
Kota × Marquis No. II-19-167	do				6		3	1	10
F_3	lines			35	78	27	25	2	167
H-44	rows	1931	*R*	2					2
Kota × Marquis No. II-19-167	do		*S*		1	1			2
F_4	lines		*R*	8	1	7	2		18
Do	do		*SR*		3	5	9	12	29
Do	do		*S*				1	3	4
H-44	rows	1930		14	1				15
Double Cross No. II-21-28	do			1	12	1	1		15
F_3	lines			35	54	68	26	4	187
H-44	rows	1931	*R*	3					3
Double Cross No. II-21-28	do		*R*		2				2
Do	do		*SR*		1				1
F_4	lines		*R*	26	9	12	3	2	52
Do	do		*SR*	3	13	5	3	10	34
Do	do		*S*				2	7	9
H-35	rows	1929		6					6
Marquis	do							5	5
F_3	lines			12	16	69	14	16	127

In the studies of the cross between H-44 and No. II-19-167 grown in 1930, all 10 rows of the H-44 parent were classed in the groups *R* and *R*–. Of the 10 parent rows of Kota × Marquis No. II-19-167, 6 were placed in the *R*– group, whereas 4 rows gave a preponderance of susceptible plants. This might be explained by the presence of physiologic forms that caused the epidemic in particular rows, or by environmental conditions in the field that caused more severe infection in some rows than in others. Of 167 F_3 lines selected at random, 35 were placed in the resistant group. If the correct classification of the *R* and *R*— lines is in a ratio of 4 to 1, as in H-44, 8 or 9 more lines might be considered as breeding true for resistance, making a total of approximately 44 out of 167 for this group. This is a ratio of about 1 homozygous resistant to 3 susceptible. These results may be explained on the basis of a single-factor difference for the H-44 type of mature-plant resistance.

Two rows of each of the parents H-44 and No. II-19-167 were grown in the rust nursery in 1931. Both rows of H-44 were placed in the *R* class, whereas the progeny of two plants of No. II-19-167, classed in 1930 as *S*, were placed in *R* in one case and in *H* in the other. Various categories of F_3 plants were studied in F_4, the selections being made from segregating F_3 lines. The progeny of *R* plants in 1931 produced homozygous *R* lines and lines classed in all other groups except the *S* group. The *SR* plants produced no lines that bred true for the *R* group and only 3 for the *R* - group; other lines were classed in the *H*, *H* , and *S* groups. One of the progenies of the 4 susceptible plants was placed in the *H*— class and 3 in the *S* class.

An analysis was made also of the data from the cross of H-44 with Double Cross No. II-21-28. Apparently one factor may explain the H-44 type of reaction in this cross. While no attempt has been made in these studies to determine the number or nature of factors contributed by the Double Cross parent, the possible relation of such factors to those governing the mature-plant resistance of H-44 has been considered.

In the cross H-35×Marquis it is impossible to say how many factors are involved for the H-35 type of reaction. Considering the proportion of heterozygous lines it seems probable that the results cannot be explained satisfactorily on a single-factor basis. Studies made previously in Minnesota and at the Dominion Rust Laboratory at Winnipeg, Manitoba, indicate that several genetic factors are responsible for the mature-plant resistance of the Kota type and of the Minnesota double crosses.

REACTION TO LEAF RUST

Data on the reaction to leaf rust of the parents and the F_3 lines of two wheat crosses are presented in table 2. From these data it is apparent that segregation has occurred. It appears rather easy to recover the type of resistance of the H-44 parent. No definite conclusion can be made regarding the number of genetic factors involved. If the two crosses are considered together, it is seen that, of 354 F_3 lines grown from random selections of F_2 plants, 28 lines were grouped as *R* and 47 lines as *R*—. It appears that only one or two factor pairs are necessary to explain the results. Little is known of the causes of mature-plant resistance to leaf rust or of the part played by physiologic forms in producing the results reported in this paper.

TABLE 2.—*Leaf-rust reaction in parent rows and F_3 lines of H-44×(Marquis×Kota No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

Parent or generation		Number of rows or lines showing indicated reaction					
		R	*R*−	*H*	*H*−	*S*	Total
H-44	rows	4	3	2	1		10
Marquis×Kota No. II-19-167	do		1		3	6	10
F_3	lines	11	28	78	22	28	167
H-44	rows	9	5	1			15
Double Cross No. II-21-28	do			2	1	12	15
F_3	lines	17	19	115	16	20	187

REACTION TO BUNT

The data on the reaction to bunt of the parent rows and F_3 lines of two wheat crosses are presented in table 3.

TABLE 3.—*Bunt reaction in parent rows and F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

Parent or cross		Number of rows or lines showing indicated percentage of bunt infection							
		0	0.1-3	3.1-6	6.1-9	9.1-12	12.1-15	15.1-18	Total
H-44	rows	8	1						9
Kota×Marquis No. II-19-167	do		5	3	1		1		10
F_3	lines	79	55	17	7	5	1	2	166
H-44	rows	14	1						15
Double Cross No. II-21-28	do	2	4	4	3	2			15
F_3	lines	121	45	11	6	3	1		187

H-44 appears to be highly resistant. Of the 24 rows grown, 22 had no bunt and 2 had only 0.1 to 3 percent. From the 2 crosses 353 lines were grown, and of these 200 had no bunt. The bunt infection was light in the parents as well as in the F_3 lines, and as 2 of the 15 lines of No. II-21-28, showed no bunt, it seems impossible to reach any conclusion regarding the inheritance of bunt reaction.

REACTION TO BLACK CHAFF

Little is known about the conditions necessary for black-chaff infection, and consequently it is difficult to obtain a satisfactory epidemic under field conditions. Much variability in infection is found in different parts of the same nursery or in fields relatively close together. An attempt was made to induce an epidemic in the cereal pathology garden, but a more satisfactory epidemic was obtained without artificial means in the stem-rust and leaf-rust nurseries. The data obtained under these conditions are presented in table 4.

Table 4.—*Reaction to black chaff in parent rows and F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown in 1930, and of H-35×Marquis, grown in 1929, at University Farm, St. Paul, Minn.*

Parent or generation		Year	Number of rows or lines included in indicated black-chaff class							Total
			1	2	3	4	5	6	7	
H-44	rows	1930			3		5	1	1	10
Kota × Marquis No II-19-167	do.						1		9	10
F_3	lines			1	5	4	69	19	69	167
H-44	rows		1	1	2	4	5	0	2	15
Double Cross No II-21-28	do							1	14	15
F_3	lines		1	19	15	45	41	19	47	187
H-35	rows	1929		3	1	2				6
Marquis	do						1	1	4	6
F_3	lines			5		93	23	3	3	127

The variable infection of H-44 in 1930 emphasizes the difficulty of the study. Thus, of 25 rows 3 showing only a trace of infection were placed in class 7, although it is known that in general H-44 is highly susceptible. It is apparent that segregation occurred in F_2 and that a large percentage of the F_3 lines was as free from infection as Kota×Marquis No. II-19-167 and Double Cross No. II-21-28, the resistant parents.

Infection was more severe in 1929, when the cross H-35×Marquis was grown. Several F_3 lines appeared to be resistant and were infected not more than was Marquis.

INDEPENDENCE OF REACTION TO THE FOUR DISEASES

In an attempt to synthesize hybrids resistant to two or more diseases it is important to determine whether there is association or independence in inheritance. If there is association and if the association is due to physiologic causes and is dependent upon the same genotypic basis, it would be impossible to obtain a new combination or relationship of the characters. If the association is dependent upon genetic linkage a new combination of parental characters would depend upon the closeness of such linkage; that is, upon the frequency of crossing-over of the genes concerned.

Table 5 shows the crosses studied, the number of F_3 lines, the character pairs studied, χ^2 for independence, *n* (degrees of freedom), and *P*. It is apparent that reaction to the following pairs of diseases is inherited independently or that the linkage relation is very loose: Stem rust and bunt; leaf rust and bunt; leaf rust and black chaff; black chaff and bunt. Stem rust and leaf rust appear to be distinctly associated. In the two comparisons of the reaction to stem rust and leaf rust, the values of *P* for the two crosses with H-44 were 0.03 and 0.05. In the comparison of the reaction to black chaff and stem rust the values of *P* were 0.05, less than 0.01, and 0.11. These were the only *P* values obtained that indicated a linkage in inheritance.

It is interesting to observe the contingency surfaces for those cases in which there appeared to be an association for reaction to two diseases. In the crosses in which H-44 was used as one parent, the relationship for reaction to stem rust and black chaff is shown in table 6 and that for stem rust and leaf rust in table 7.

TABLE 5.—*Independent inheritance of reaction to various diseases in the F_3 lines of wheat crosses grown at University Farm, St. Paul, Minn., 1930*

Cross	Number of F_3 lines	Characters compared	χ^2	n	P
H-44×Kota×Marquis No. II-19-167	167	Stem rust and leaf rust	18.56	9	0.03
H-44×Double Cross No. II-21-28	187	do	12.81	6	.05
H-44×Kota×Marquis No. II-19-167	166	Stem rust and percentage of bunt	11.85	9	.22
H-44×Double Cross No. II-21-28	187	do	7.52	6	.27
H-44×Kota×Marquis No. II-19-167	167	Stem rust and black chaff	16.81	9	.05
H-44×Double Cross No. II-21-28	187	do	41.53	9	(a)
H-35×Marquis	127	do	4.15	2	.11
H-44×Kota×Marquis No. II-19-167	166	Leaf rust and percentage of bunt	7.26	9	.61
H-44×Double Cross No. II-21-28	187	do	7.15	4	.09
H-44×Kota×Marquis No. II-19-167	167	Leaf rust and black chaff	10.29	9	.33
H-44×Double Cross No. II-21-28	187	do	6.60	6	.37
H-44×Kota×Marquis No. II-19-167	166	Black chaff and percentage of bunt	3.37	9	.50
H-44×Double Cross No. II-21-28	187	do	8.09	6	.24

[a] Less than 0.01.

TABLE 6.—*Reaction to stem rust and black chaff in F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

H-44×(KOTA×MARQUIS NO. II-19-167) [a]

Black-chaff class	Number of F_3 lines showing indicated reaction to stem rust: *R*	*R*−	*H*	*H*−, *S*	Total
2, 3, 4	5	3	2		10
5	16	30	14	9	69
6	5	9	4	1	19
7	9	36	7	17	69
Total	35	78	27	27	167

H-44×DOUBLE CROSS NO. II-21-28 [b]

Black-chaff class	*R*	*R*−	*H*	*H*−, *S*	Total
1, 2, 3	18	5	9	3	35
4	6	15	21	3	45
5	5	10	19	7	41
6, 7	6	24	19	17	66
Total	35	54	68	30	187

[a] $P=0.05$.

[b] P=less than 0.01.

TABLE 7.—*Reaction to stem rust and leaf rust in F_3 lines of H-44×(Kota×Marquis No. II-19-167) and H-44×Double Cross No. II-21-28, grown at University Farm, St. Paul, Minn., 1930*

H-44×KOTA×MARQUIS NO. II-19-167 [a]

Reaction to leaf rust	Number of F_3 lines showing indicated reaction to stem rust: *R*	*R*−	*H*	*H*−, *S*	Total
R, *R*	9	20	5	5	39
H	21	32	16	9	78
H	3	7	5	7	22
S	2	19	1	6	28
Total	35	78	27	27	167

H-44×DOUBLE CROSS NO. II-21-28 [b]

Reaction to leaf rust	*R*	*R*−	*H*	*H*−, *S*	Total
R, *R*−	13	9	11	3	36
H	18	30	46	21	115
H−, *S*	4	15	11	6	36
Total	35	54	68	30	187

[a] $P=0.03$.

[b] $P=0.05$.

It is apparent that linkage is in the same direction as in the parent varieties, that is, there is a preponderance of lines resistant to stem rust, susceptible to black chaff, and resistant to both stem rust and leaf rust. However, in the stem-rust-resistant lines the proportion of lines in black-chaff classes 6 and 7 (resistant) is much greater in the F_3 hybrids than in the H-44 parent, which leads to the belief that lines resistant to the three diseases can be obtained from crosses in which H-44 is used as one parent.

SUMMARY

Correlated reaction in wheat to stem rust, leaf rust, bunt, and black chaff was studied in the F_3 progeny of crosses between H-44 with Double Cross No. II-21-28 and Kota × Marquis No. II-19-167, respectively. The F_3 lines were grown from random selections of F_2 plants.

Epidemics of stem rust, leaf rust, and bunt were created artificially, but black chaff developed naturally in the rust nurseries.

Inheritance of stem-rust resistance of the mature-plant type of the H-44 parent appeared to be dependent upon a single genetic factor difference. The moderate plant resistance of Nos. II-19-167 and II-21-28 appeared to be dependent upon factors not allelomorphic to those determining mature-plant resistance of the H-44 type, as susceptible lines were obtained in the F_3 generation. There was some indication that more than a single-factor pair was necessary to explain the stem-rust resistance of mature plants of the H-35 parent in the crosses with Marquis.

It was impossible to determine the number of factor pairs responsible for segregation of reaction to leaf rust, bunt, and black chaff, although a considerable number of resistant types were obtained in all cases.

Independence of reaction to all combinations of stem rust, leaf rust, bunt, and black chaff was studied by the use of χ^2 for independence.

The inheritance of reaction to the following combinations of diseases appeared to be independent: Stem rust and bunt, leaf rust and bunt, leaf rust and black chaff, and black chaff and bunt.

There appeared to be linkage in the inheritance of reaction to stem rust and leaf rust and of reaction to stem rust and black chaff However, a comparison of the F_3 reactions with those of the H-44 parent indicates the possibility of combining the mature-plant resistance to stem rust of the H-44 type with resistance to black chaff of many varieties and hybrids of common wheat.

EFFECT OF WASHING ON THE KEEPING QUALITY OF HENS' EGGS[1]

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INTRODUCTION

Under the ordinary system of poultry management, a considerable percentage of the eggs gathered are dirty. Such eggs bring a lower price than naturally clean ones because of their insanitary appearance and poor keeping quality If the dirty eggs are cleaned by washing, the dirt is removed but the keeping quality is not improved; according to current ideas, it is materially lessened.

The explanation most frequently offered to account for the belief that washed eggs do not keep so well as unwashed clean ones is that washing removes a film from the surface of the eggs which acts as a protective coating. Its removal is supposed to open the pores of the shell, thus permitting the entrance of micro-organisms which cause spoilage, and accelerating the escape of water, which results in shrinkage or loss in weight of the egg during marketing and storage.

The work herein recorded was undertaken to determine the effect on the keeping quality of eggs of washing and of the type of solution used in washing. As an incidental part of the study a few comparisons were made between cleaning by washing and by dry abrasion.

LITERATURE

The general prevalence of dirty eggs is indicated by several investigators.

In an investigation covering 3 years, Huttar (*1*)[2] found that among the eggs produced by the Cornell poultry flock, the percentage of dirty eggs ranged from 9.8 in July to 24.6 in March and averaged 17.7. He reports:

> The flocks from which these eggs were gathered are managed about the way that the average commercial poultryman manages his flock.

Van Wagenen (*32*) studied the effect of nesting material and litter on the production of dirty eggs. He found that the percentage of dirty eggs varied from 77 for the experiment with straw litter and no nesting material to 23.2 with straw litter and shavings for nesting material.

Pennington and Pierce (*20*) examined a large number of eggs in the New York market. In one series of 258,496 dozen they found 12.58 percent dirty and in another series of 238,446 dozen they found 13.40 percent dirty. Perhaps some of the eggs which they classified as clean were actually washed dirty eggs.

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[1] Received for publication Mar. 1, 1933, issued February 1934. The major part of this paper was taken from a thesis presented by the senior writer to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy, June 1928.

[2] Reference is made by number (italic) to Literature Cited, p. 87.

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Sharp (*26*) examined 30 samples of eggs of the dozen each obtained from lots offered for sale in a city of up-State New York. Using special tests for detecting the cleaned eggs, he found that 39 percent of the eggs were either cleaned or dirty.

Some of the factors which affect the loss in weight of eggs have been studied. In 1908 Cook, in a report by Wiley et al. (*33*), stated that eggs in storage for 7.5 months sustained a loss in weight equivalent to 5.4 percent of the total weight, which is largely water from the whites. Through improved control of storage conditions the loss in weight is now about 3 to 5 percent for a storage period of about 8 months.

Greenlee (*10*) pointed out that water also passes from the white to the yolk. In his experiments he found that the loss in weight may be described by a parabolic curve, the rate of loss increasing with temperature and decreasing with time. Neither Cook nor Greenlee indicate whether they kept their eggs in an environment of constant humidity.

Dunn (*8, p. 55*) stated:

> If we regard the rate at which an egg loses weight as an expression of the permeability of the envelopes which enclose it, and especially the porosity of the shell, then the shells of eggs must be more variable than the sizes of the eggs themselves.

Dunn (*9*) determined the correlation between the fresh weight and the percentage loss in 2 lots of eggs and obtained the values $r = -0.331 \pm 0.040$ and $r = -0.357 \pm 0.068$. When the fresh weight was correlated with the absolute loss, the value was $r = +0.187 \pm 0.044$. The larger the eggs the greater was the absolute loss but the smaller the percentage loss. Considering the rate of evaporation per unit surface, Dunn concluded (*9, p. 170*): "It is legitimate to assume * * * that the shells of larger eggs are somewhat less porous and tend to conserve moisture better than the shells of smaller eggs."

Curtis (*7*) and Jull (*16*) found that the weight of the shell was quite variable as compared with other parts of the egg.

The conditions under which bacteria will grow in egg white and shell eggs have received considerable attention. Wurtz (*34*), Scholl (*24*), Laschtschenko (*17*), Rettger and Sperry (*22*), and others have shown that egg white has germicidial properties. Healy and Peter (*13*) and Sharp and Powell (*27*) reported that the hydroxyl-ion concentration of the white of eggs increases very rapidly after the eggs are laid, owing to the loss of carbon dioxide, and approaches as a limit pH 9.5-9.7. Sharp and Whitaker (*29*) and Stark and Sharp (*30*) demonstrated that the germicidal action of the egg white is influenced markedly by this change in hydroxyl-ion concentration.

Pennington (*19*) and Stiles and Bates (*31*) found that a considerable number of fresh eggs contained bacteria, although the bacterial count in most cases was low. They plated the material as soon as the eggs were opened. Since others were skeptical because this method yielded so many sterile eggs, they separated the white and yolk and incubated the yolk in a flask for some time before plating. Using this method, Maurer (*18*) found bacteria in 22.9 percent of one lot of 292 fresh eggs and in 25.4 percent of another lot of 283 eggs. Bushnell and Maurer (*6*) found that 23.7 percent of 275 eggs examined contained bacteria, and Hadley and Caldwell (*12*) found that of 2,500 fresh eggs examined 8.7 percent contained bacteria. This method of separating the yolk and allowing it to incubate for some time before plating has the very serious disadvantage that the yolk may become contaminated durings its separation from the egg and introduction into the flask.

The study of the bacteriology of fresh eggs by Rettger (*21*) deserves especial consideration. The eggs which he examined were normal clean ones. After incubating the whole yolk from 3,510 fresh eggs he found 9.5 percent infected. Of 647 eggs in which he pipetted 10 cc of yolk for a sample for incubation, the infection was 3.86 percent. He says: "Even 3.9 percent is, in all probability, considerably above the actual figures, could accidental invasion of bacteria in the tests be entirely prevented."

If bacteria are present in fresh eggs, the number should be increased by incubation. Rettger incubated 1,746 eggs for 7 to 10 days and found 2.75 percent infection; 2,166 eggs for 2 weeks and found 1.3 percent infection; and 1,984 eggs for 3 weeks and found 3.6 percent infection.

The following statements by Rettger are so significant in relation to the problem treated in this paper that they are quoted (*21, pp. 207, 208, 210*):

> It is impossible to find a satisfactory explanation of the unqualified success which is attained with some of the strongly advocated processes for preserving or pickling eggs, unless it is assumed that sound, fresh eggs are, as a rule, sterile * * *
>
> Hence, the only safeguard of the contents of the egg against bacterial decomposition from within is a state of absolute asepsis or sterility at the outset * * *
>
> The soiling of shells, especially with fecal matter, lowers the chances of preservation of the contents. Eggs which are soiled are no longer germproof.

Jenkins, Hepburn, Swan, and Sherwood (*14*) reported that they found bacteria in few naturally clean eggs after storage; bacterial infection was greater in stored dirty eggs and greater still in stored washed dirty eggs.

Jenkins and Pennington (*15*) found that dirty eggs showed 12 to 30 bad eggs per case before the candle and 10.5 to 29 additional on breaking the eggs. They stated that it was not always possible to recognize washed eggs.

METHODS

The factor most often used in judging the quality of eggs is the size of the air cell, since it can be seen clearly in the candling test. Since the increase in size of the air cell is due to evaporation of water from the egg, the loss in weight of the egg becomes the most accurately measurable factor in determining the effect of the various treatments on evaporation. The number of pores in the shells was correlated with the loss in weight of the eggs as a possible explanation of the individual variations that were found.

During storage, water passes from the white of the egg to the yolk, and consequently the percentage of total solids in the yolk decreases. The percentage of total solids in the white increases as a result of the passage of water to the air and to the yolk. A determination of the total solids in the white and yolk is, therefore, an aid in determining changes in quality.

The ability of the yolk to stand up in a dish after the egg is broken is a quality factor which is readily expressed numerically by dividing the height by the width. The better the quality of the yolk, the higher the value of this quotient (*28*).

The hydrogen-ion concentration of the white and yolk was determined as an indication of the storage conditions (*25*).

If bacteria grow abundantly in the egg, it deteriorates rapidly in quality. Since in washing eggs (especially dirty ones) bacteria may be rubbed through the pores of the shell, a comparison of the bacteria found in the white and yolk of the eggs subjected to the various treatments was made.

Clean as well as dirty eggs were washed. By washing clean eggs, the influence of the actual washing itself could be studied without the complicating factor of dirt on the shell.

The eggs, after being subjected to the different treatments, were stored either at 35° C. or at room temperature, 20° to 25°. This relatively high temperature was chosen because at higher temperatures deterioration is more rapid and thus the time required for the experiments was shortened.

Since eggs lose water at different rates, depending on the humidity of the air in which they are stored, the eggs were kept in air of constant humidity. This was accomplished by covering the bottom of a closed container with a saturated sodium chloride solution containing an excess of undissolved sodium chloride. The eggs were supported on a wire tray about 3 inches above the surface of the solution. The container was large enough to permit the eggs of an entire experiment to be placed in a layer one egg deep. The humidity at the surface of the solution was approximately 75 percent.

The eggs used were laid by the flocks of the Poultry Department of Cornell University during 1927–28. They were infertile eggs, produced by White Leghorn pullets. The eggs were put through the various treatments and placed in storage the day they were laid (An exception in the case of the extremely dirty eggs will be noted later.) No less than 6 eggs, and frequently 12, were subjected to each treatment in an experiment. At stated intervals during the storage period, each egg was weighed to the nearest milligram On the twentieth day they were candled, and the apparent pores or spots which could be seen before the candle were counted on 1 cm^2.

At the end of the storage period, 27 to 30 days, the following factors were determined: (1) Number of pores that gave bubbles when the egg was immersed in water and placed under a vacuum; (2) breaking strength of the shell; (3) presence of bacteria in the white; (4) presence of bacteria in the yolk; (5) height and width of the yolk; (6) total solids in the white; (7) total solids in the yolk; (8) pH value of the white; and (9) pH value of the yolk.

When the eggs were to be cleaned with water or a solution, they were immersed for about a minute in a container sufficiently large to hold both liquid and eggs. Each egg was then scrubbed with a fairly stiff brush, rinsed in tap water, and placed on a rack to dry. The cleaning treatments, selected because of some special property of the solution, such as acidity, alkalinity, bactericidal effect, or abrasiveness, were as follows: (1) Control eggs stored without treatment; (2) eggs washed with distilled water; (3) washed with N/10 sulphuric acid; (4) washed with N/10 sodium hydroxide; (5) washed with a water-glass solution, prepared by diluting 1 volume of commercial sodium silicate, used for preserving eggs, with 12 volumes of water; (6) washed with a soap solution prepared by dissolving 50 g of white naphtha soap in 3 l of water; (7) washed with a fairly strong solution of Wyandotte alkaline washing powder; (8) soaked in water, dusted with powdered Sapolio, scrubbed with a brush, and rinsed; (9) washed with a hypochlorite solution prepared by dissolving 1 part of a commercial hypochlorite powder in 100 parts of water; (10) cleaned with sandpaper; (11) dipped for 3 seconds (without previous treatment) in oil that had been heated to 125° C.; (12) washed with distilled water and oil-dipped; (13) sandpapered and oil-dipped; (14) washed with soap and oil-dipped; (15) washed with hypochlorite solution and oil-dipped.

EXPERIMENTAL DATA

FACTORS AFFECTING LOSS IN WEIGHT OF EGGS

It was found that the eggs lost about 0.025 g each after being washed and dried in air. Just how much of this loss was due to the removal of material from the surface of the egg, and how much to the evaporation of water during the drying, is not known.

Method of Cleaning

To determine the loss in weight of eggs cleaned by different methods and held at different temperatures, the following experiments were made:

1. Clean eggs, stored in cartons, holding temperature 35° C.—Naturally clean eggs were gathered, washed, dried, and weighed on the day they were laid. They were then placed in cartons holding 1 dozen each, and held for 27 days at 35° C. in a closed container over a saturated solution of sodium chloride. One dozen eggs were subjected to each treatment. Each egg was weighed at 3-day intervals. It was thought that possibly by retarding diffusion, the cartons might have obscured the effect of the washing. Therefore in subsequent experiments the eggs were placed in a layer one egg deep on a woven-wire tray suspended about 3 inches above the surface of the sodium chloride solution.

2. Clean eggs, holding temperature 35° C.—The procedure was the same as in 1, except that the eggs were not stored in cartons and were weighed at 6-day intervals for 30 days.

3. Extremely dirty eggs, holding temperature 35° C.—A compost of hen manure and a bouillon culture of *Pseudomonas pyocyanea* were made up to a pasty consistency. Fresh, clean eggs were dipped in the compost, allowed to dry overnight, and washed the following day. The eggs were made very dirty, so that extreme conditions might be studied. The weighing was discontinued in practically every instance because the gas pressure created by bacterial infection within the egg caused the contents to spew through the pores, resulting in abnormal decreases in weight. Six eggs were subjected to each treatment.

4. Naturally dirtied eggs, holding temperature 35° C.—Eggs very dirty when gathered from the nest were subjected in lots of six eggs each to the different cleaning procedures and then held at 35° for 30 days.

5. Clean eggs, holding temperature 20° to 25° C.—Naturally clean eggs in lots of six eggs each were cleaned as indicated, and stored for 27 days at room temperature, 20° to 25°, over a saturated solution of sodium chloride.

6. Extremely dirty eggs, holding temperature 20° to 25° C.—The procedure was the same as in 3. The spewing was less pronounced, however, and the weighings were sufficiently reliable to be presented in table 1. The comparatively large loss in weight of the unwashed dirty eggs was due to the mechanical loss of material from the surface of the shell.

7. Naturally dirty eggs, holding temperature 20° to 25° C.—This experiment was similar to 4, except for the holding temperature.

The percentage loss in weight of the various lots is given in table 1, and the actual loss in weight in grams for the various time intervals is given in figures 1 to 6. The figures indicate a linear relation between loss in weight and time during the period studied. This is noted especially at 35° C., where the temperature control was better.

No definite effect of washing on the loss in weight is indicated. A definitely retarding effect of oil-dipping on the rate of loss in weight is shown.

The size of the air cells was measured after the eggs had been held for 20 days. The eggs that lost the least weight had the smallest air cells. The eggs held at 35° C. had larger air cells than those held at room temperature. No effect of washing was indicated.

TABLE 1.—*Average percentage loss in weight of eggs cleaned by various methods and cleansing agents*

Cleansing agent and treatment	Clean eggs washed and held at 35° C.		Naturally dirtied eggs washed and held at 35° C. 30 days	Eggs washed and held at room temperature, 20°-25° C.		
	27 days, in cartons	30 days		27 days (clean eggs)	30 days	
					Extremely dirty eggs	Naturally dirtied eggs
Water	6.67	7.60		2.56	2.75	
H_2SO_4, N/10	5.57	6.81		2.83	3.28	
NaOH, N/10	5.96	7.21		2.90	3.10	
Water glass	6.02	6.78		2.68	3.69	
Water, oiled	.53	.55		.31	.59	
Oiled only	.65	.53	0.58	.41		0.19
Soap	6.21	7.15	6.77	2.80	3.08	4.81
Wyandotte powder	5.42	7.20		2.95	3.44	
Sapolio	6.69	7.15		2.98	3.21	
Hypochlorite			6.87		3.88	4.42
Sandpaper			6.33		4.28	4.18
Hypochlorite, oiled			.81			.28
Sandpaper, oiled			.54			.70
Soap, oiled			.45			.34
Dirty, unwashed			6.64		5.01	4.77
Clean, unwashed	6.42	7.48		3.22	3.39	

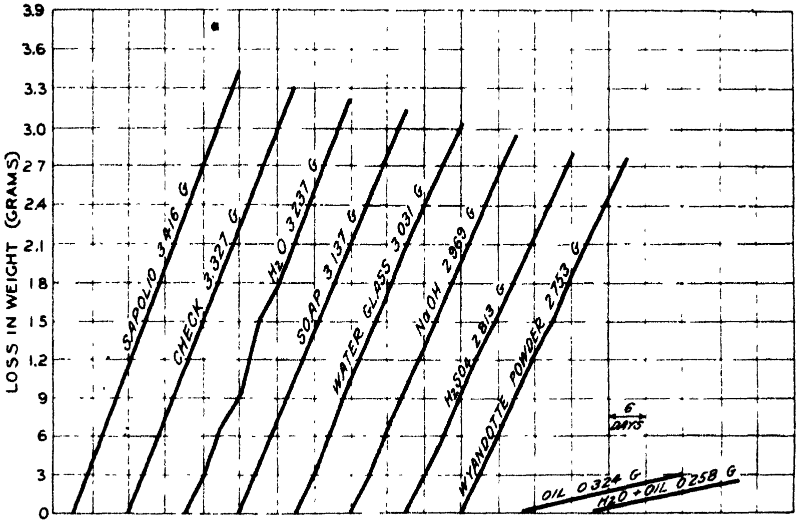

FIGURE 1.—Loss in weight (grams) of clean eggs after being washed with various solutions and held in cartons at 35° C. and 75 percent humidity for 27 days.

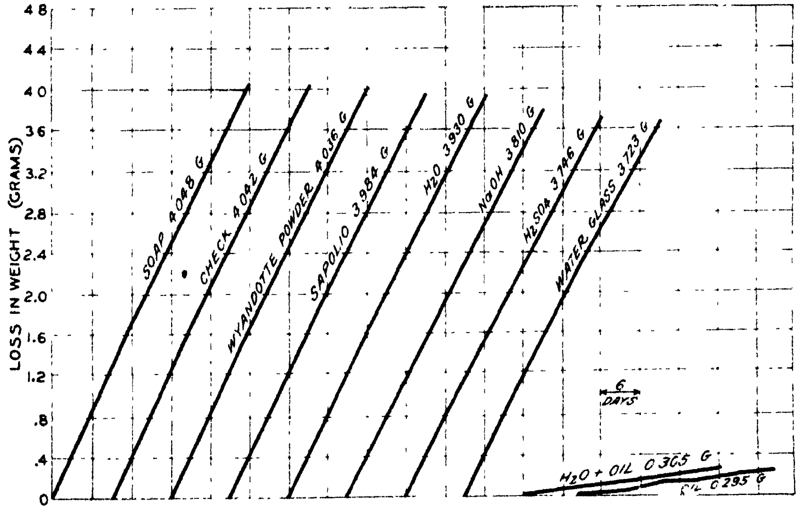

FIGURE 2.—Loss in weight (grams) of clean eggs after being washed with various solutions and held at 35° C. and 75 percent humidity for 30 days.

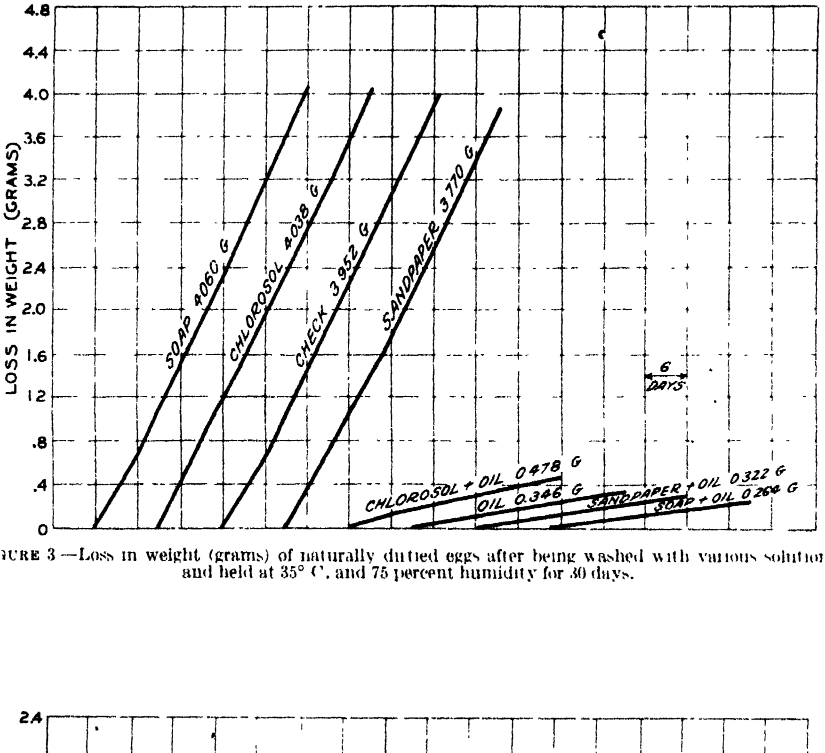

FIGURE 3.—Loss in weight (grams) of naturally dirtied eggs after being washed with various solutions and held at 35° C. and 75 percent humidity for 30 days.

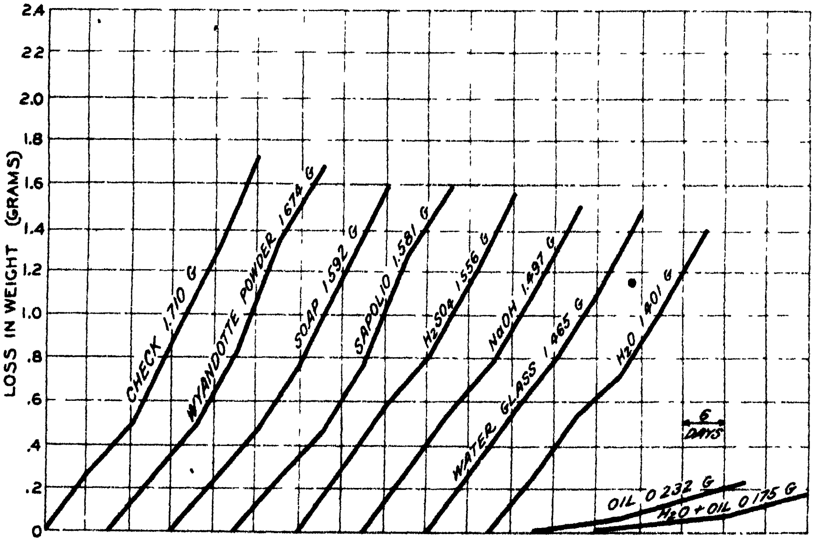

FIGURE 4.—Loss in weight (grams) of clean eggs after being washed with various solutions and held at room temperature (20°–25° C.) and 75 percent humidity for 30 days.

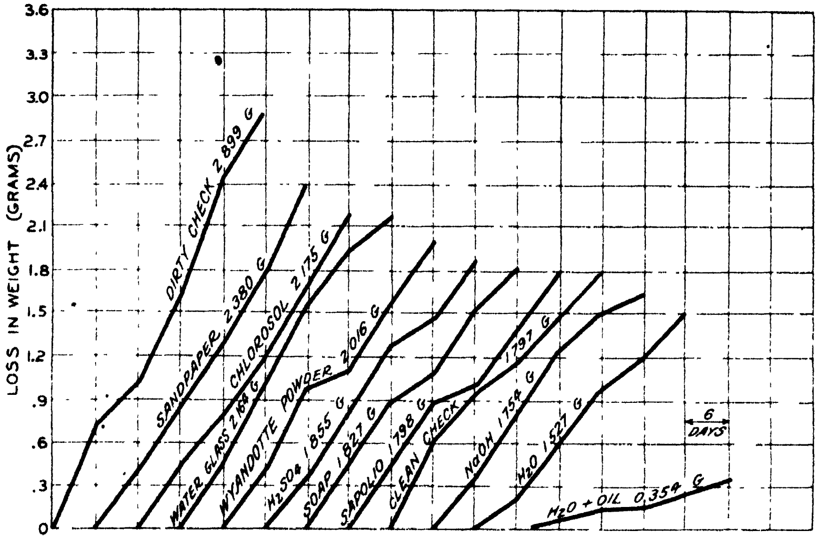

FIGURE 5 — Loss in weight (grams) of extremely dirty eggs after being washed with various solutions and held at room temperature (20°-25° C.) and 75 percent humidity for 30 days

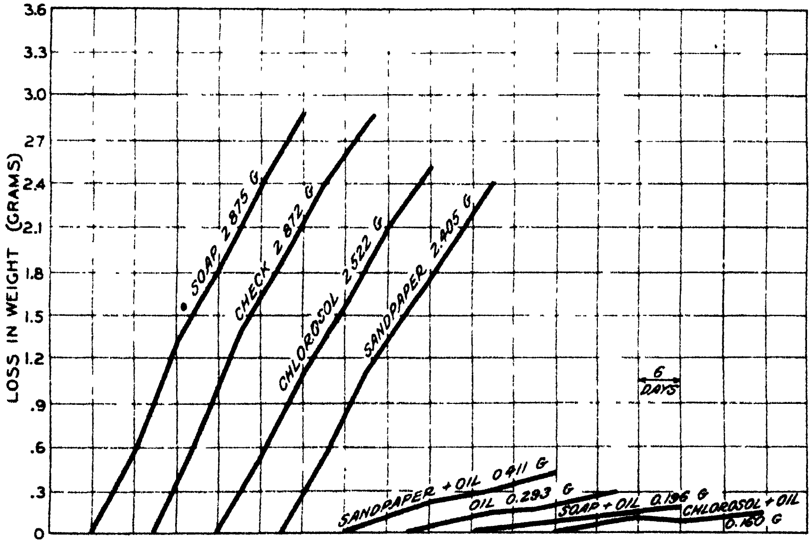

FIGURE 6 — Loss in weight (grams) of naturally dirtied eggs after being washed with various solutions and held at room temperature (20°-25° C.) and 75 percent humidity for 30 days

Cold Storage

A case of eggs was placed in an egg-storage room of a commercial cold-storage warehouse. The case contained naturally clean eggs, naturally clean eggs which had been washed, naturally dirty eggs, and naturally dirty eggs which had been washed. Each egg was weighed at the beginning of the experiment and after 4.5 and 13.5 months in storage. The mean loss in weight and the probable error for each lot follow:

Storage 4.5 months:	
87 clean eggs	2.317 ± 0.043
88 clean eggs washed	2.315 ± .036
83 dirty eggs	2.416 ± .037
89 dirty eggs washed	2.365 ± .033
Storage 13.5 months:	
84 clean eggs	5.836 ± .099
86 clean eggs washed	5.866 ± .089
82 dirty eggs	6.034 ± .099
88 dirty eggs washed	5.911 ± .086

The distribution of the eggs was varied throughout the case. This was necessary because often there is considerable variation in the loss in weight of eggs in different layers and in different sides of a case.

The conclusion seems justified that washing in itself has no effect on the rate of loss in weight of eggs in cold storage. The dirty eggs lost more weight than the washed dirty eggs. This difference is insignificant, however, and might have been due to the mechanical loss of the dirt from the eggs.

Removal of Film from Surface of Shell

Eggs were dipped in 10-percent solutions of nitric, hydrochloric, or sulphuric acid for a few seconds, and the protein material thus loosened was rubbed off by washing in tap water. Ten eggs were treated with each acid, held at laboratory temperature with no humidity regulation, and weighed at various intervals. The results obtained are given in table 2. These results indicate that eggs lose weight more slowly after the film is removed. Since the calcium salts formed by the action of nitric and hydrochloric acid on the shell are very soluble, a plugging action due to the formation of insoluble inorganic salts would hardly be expected. Thus it must be concluded that washing eggs does not remove the protecting film from the surface of the shell (*26*), and that if it did the eggs would not lose moisture faster than if it were present.

The results presented in table 2 have been confirmed by other experiments. It seems possible that the material which was removed from the surface of the egg might, when present, act as the outer end of a capillary wick passing between the crystals of the shell to draw moisture from the egg and to increase the evaporating surface.

TABLE 2.—*Effect of washing eggs with 10 percent solutions of nitric, hydrochloric, and sulphuric acids on the percentage loss in weight when held at 20°–25° C. for varying periods*

[This treatment removes a film from the surface of the egg Each value is the average of the results obtained with 10 eggs]

Time (days)	Percentage loss in weight of eggs: Not washed	Washed with— HNO_3	Washed with— HCl	Washed with— H_2SO_4	Time (days)	Percentage loss in weight of eggs: Not washed	Washed with— NHO_3	Washed with— HCl	Washed with— H_2SO_4
3	1.26	1.12	1.10	1.13	14	4.74	4.31	4.29	4.39
6	2.12	1.90	1.88	1.94	21	6.73	6.20	6.18	6.26
11	3.72	3.35	3.35	3.44	32	9.65	9.02	8.95	8.92

USE OF THE SAND BLAST

For some years the sand blast has been utilized for cleaning eggs. This method does not involve the wetting of the shell. In some of the experiments described above, eggs were cleaned with sandpaper in an attempt to simulate cleaning with a sand blast. Contrary to expectation, the sandpapered eggs did not differ greatly in rate of loss of water from those that were not sandpapered. This result was obtained in spite of the fact that the sandpapering increased the number of pores, as determined by the water-vacuum method.

Some information as to the effect of the commercial sand blast on the number of pores and the loss in weight of eggs was obtained. Two dozen eggs which had been through the sand blast and 2 dozen similar eggs which had not been sand-blasted were kept in the laboratory at 20° to 25° C., with no humidity regulation, for 4 weeks. The eggs were weighed individually at weekly intervals. The experiment was carried out in the winter, when the humidity of the laboratory was rather low. At the end of 4 weeks the number of pores, as indicated by the appearance of a continuous stream of bubbles when the eggs were immersed in water and a suction pump applied, was determined. The average number of pores on the eggs which were not sand-blasted was 18, while the average number on the sand-blasted eggs was 53. Most of the pores on the sand-blasted eggs were small, however, if the rate of escape of air may be taken as an indication of size. Table 3 shows the average loss in weight of the two lots of eggs.

TABLE 3.—*Percentage loss in weight of sand-blast-treated eggs as compared with untreated eggs when held at 22°–25° C. for from 1 to 4 weeks*

Time (weeks)	Percentage loss in weight of eggs: Not sanded	Percentage loss in weight of eggs: Sanded	Increase in loss in weight due to sanding
1	2.90	3.54	22.05
2	5.78	6.93	20.12
3	8.14	9.66	18.7
4	11.23	13.18	17.4

The sand-blasted eggs lost weight faster than the eggs that were not sand-blasted, but the average increase in the loss in weight was only about 20 percent, whereas the increase in the number of pores was over 200 percent. Evidently some other property exerted a strong influence on the loss in weight.

This experiment was repeated 2 years later, with essentially the same results. Eggs collected from a single pen of hens were divided into three lots of 2 dozen each, and held for 45 days after treatment at a temperature of 25° to 30° C. and a relative humidity of 75 percent. In this experiment the sanded eggs were put through the sanding machine twice. The losses in weight were as follows: Control, no treatment, 9.4 percent; dirty and sanded, 13.1 percent; clean and sanded, 13.6 percent.

NUMBER OF PORES AND STRENGTH OF SHELL

As it was believed that the number of pores in the shell might affect the loss in weight, the pores were counted in the eggs used for the data in table 1. These pores were determined by immersing an egg in water, creating a vacuum, and counting the sources of bubbles that came from the egg. The average number of pores for each lot in the various experiments is given in table 4.

TABLE 4.—*Average number of pores in shells of eggs listed in table 1 that were cleaned by various methods and cleansing agents*

[Determined by evacuating eggs under water]

Cleansing agent and treatment	Clean eggs washed and held at 35° C: 27 days, in cartons	Clean eggs washed and held at 35° C: 30 days	Eggs washed and held at 35° C for 30 days: Extremely dirty eggs	Eggs washed and held at 35° C for 30 days: Naturally dirtied eggs	Eggs washed and held at room temperature, 20°–25° C: 27 days (clean eggs)	Eggs washed and held at room temperature, 20°–25° C: 30 days: Extremely dirty eggs	Eggs washed and held at room temperature, 20°–25° C: 30 days: Naturally dirtied eggs
H_2O	15	13			10	10	
H_2SO_4 N/10	8	8			8	9	
NaOH N/10	13	22			21	14	
Water glass	6	13			8	11	
Water, oiled	5	5	5		3	4	
Oiled	4	3		7	6	5	7
Soap	7	9		18	11	5	21
Wyandotte powder	6	8			8	11	
Sapolio	9	17			14	10	
Hypochlorite				22		20	17
Sandpaper			46	28		37	12
Hypochlorite, oiled				8			11
Sandpaper, oiled				10			6
Soap, oiled				9			10
Dirty, unwashed				13		18	10
Clean, unwashed	5	7	9		10	13	

Although the results are not wholly consistent, a few points are indicated. The eggs cleaned with sandpaper had more pores than any of those cleaned by other means. In the lots cleaned with sulphuric acid the number of pores was about the same in each experiment, whereas in the other lots the number of pores varied widely in the different experiments. In these experiments the temperature at which the eggs were held had no effect on the number of pores.

To determine whether the various treatments had any effect upon the strength of the shell the apparatus described by Romanoff (*23*) was used. The smallest weight required to crack the shell of each egg was determined. The average of the weights necessary to break the shells in each lot of the various experiments is given in table 5.

TABLE 5.—*Average weight necessary to break shell of eggs listed in table 1 that were cleaned by various methods and cleansing agents and held for 30 days*

[Breaking strength in kilograms]

Cleansing agent and treatment	Eggs washed and held at 35° C			Eggs washed and held at room temperature, 20°-25° C		
	Clean	Extremely dirty	Naturally dirtied	Clean	Extremely dirty	Naturally dirtied
H_2O	5.1			5.5	3.7	
H_2SO_4	5.4			4.7	4.2	
NaOH N/10	5.1			5.1	4.2	
Water glass	4.1			4.7	4.6	
Water, oiled	4.7	4.7		5.4	4.7	
Oiled	4.2		4.0	4.9		4.1
Soap	5.0		4.4	4.5	4.4	4.0
Wyandotte powder	4.4			5.3	4.3	
Sapolio	4.7			4.5	4.4	
Hypochlorite			4.6		4.5	3.9
Sandpaper		4.4	4.1		4.0	4.2
Hypochlorite, oiled			3.8			4.8
Sandpaper, oiled			3.7			3.7
Soap, oiled			3.7			4.1
Dirty, unwashed			3.7		4.5	3.6
Clean, unwashed	4.2	4.1		4.1	3.6	

The results there shown indicate that washing had no effect upon the strength of the shells.

INDIVIDUAL VARIATIONS IN LOSS OF WEIGHT

In each lot studied there was always a variation in the amount of water lost by the individual eggs. Several factors which might account for this variation were studied. Since it was impracticable to divide the eggs in each lot in such a way that the average weights would be exactly the same, a study was made to determine whether the size affected the absolute loss in weight of the egg. The size of the egg, fresh weight, was correlated with the loss in weight after the egg had been held for 30 days at 35° C. The correlation coefficient was $+0.082 \pm 0.067$. Dunn (*9*) says, "There is probably a small or loose association between weight and absolute loss of weight." In the present experiment the association was so small that it is unlikely that egg size had any appreciable effect upon the losses of the various lots.

To determine the effect of the pores upon the loss in weight, correlations were made with two groups of eggs. The eggs were stored for 27 and for 30 days at 35° C. In the first group, all of the eggs used in the first experiment (of table 1) except those that were oil-dipped were considered. The correlation coefficient for the 96 eggs was $+0.450 \pm 0.057$. The second group was made up of 60 unwashed, clean eggs. The correlation coefficient was $+0.461 \pm 0.080$. Both of these are significant. It must be taken into consideration that the size of the pore probably influenced the results, and that there were doubtless many minute pores that could not be determined by the method used in this experiment.

Since there was some question as to whether the number of pores affected the strength of the shell, correlations were made upon 60 clean, unwashed eggs that had been held at 35° C. for 30 days and tested for porosity and shell strength. The correlation coefficient between shell strength and number of pores was +0.258±0.033. Although the correlation is small, it is significant and positive.

An examination of the shell by candling after they had been held for a few days at 75 percent humidity, revealed the presence of a large number of spots. It was thought that the number of spots might be indicative of the condition of the eggs, since they were not apparent when the eggs were first laid. The spots were counted on a shell area of a square centimeter at about midway between the two extremes of the egg. It was found that temperature and washing bore no relationship to these spots. In some instances there were more spots on the eggs that were held at 35° C. than on those held at room temperature. In other instances the reverse was true, and it was concluded that the treatment of the eggs did not affect the number of spots present. The number of pores was correlated with these spots. The correlation coefficient was +0.172±0.098.

FACTORS AFFECTING BACTERIAL COUNT IN EGGS

Germicidal Action of Egg White

Sharp and Whitaker (*29*) found that egg white corresponding in pH to the white of fresh eggs (7.6 to 8.7) was not germicidal, while egg white corresponding in pH to the white of aged eggs (9.5) was germicidal. All of their tests on the germicidal action of egg white were made at 37° C., and the experiment was concluded at the end of 6 hours. In the experiments reported here the germicidal action of egg white was studied at several temperatures and up to 8 days time.

A series of test tubes was arranged, containing equal volumes of well-mixed egg whites sufficient to fill the tubes to about one half of their capacity. The pH of the whites of one half of the tubes was adjusted to 7.48, and that of the other half to 9.56. One cubic centimeter of a broth culture of *Pseudomonas pyocyanea* was added to each tube. The tubes were closed with rubber stoppers in order to prevent the entrance or escape of carbon dioxide, which would have caused a change in the hydroxyl-ion concentration. Tubes containing egg white of each of the pH values were placed at temperatures of 0°, 7°, 16°, 20°, 35°, and 40° C. At various intervals a tube of each was removed, the bacteria plated, and the pH values determined. The results are shown in table 6.

From table 6 it appears that when the pH value is high, germicidal action takes place at all temperatures, but most rapidly at the higher temperatures. When, owing to the production of acid by the bacteria, the pH is lowered, the germicidal action becomes less and the organisms begin to multiply. This multiplication of bacteria began after about 1 day at the higher temperatures, and by the end of the fourth day the pH had dropped to about 6.2 at 20° and 35° C. At the end of 8 days, the bacterial count of the egg white stored at 0° was still decreasing.

At the low pH (7.48) the organisms, after a slight period of lag, multiplied at all temperatures except 0°, and were able to maintain themselves at this temperature. The pH values dropped slightly, but they did not go low enough in 8 days to affect bacterial growth materially.

TABLE 6.—*Effect of pH 9.56 and 7.48 of egg white on growth of pseudomonas pyocyanea (number per cubic centimeter) at different temperatures and at various intervals*

[000 omitted in bacteria for pH 9.56 and 000,000 for pH 7.48]

Temperature (°C)	Bacteria at start	Bacteria after— ½ hour	1 hour	2 hours	3 hours	4 hours	6 hours	8 hours	Bacteria after 1 day	pH after 1 day	Bacteria after 2 days	pH after 2 days	Bacteria after 4 days	pH after 4 days	Bacteria after 8 days	pH after 8 days
	pH 9.56															
0	2,000	---	900	1,030	---	930		1,200	290	9.57	10	9.54	7	9.54	0.4	9.65
7	2,000	---	1,090	1,590	---	910	---	1,070	610	9.43	213	9.56	112	9.52	660	9.45
16	2,000	---	1,100	1,840	---	1,115	---	1,310	790	9.43	1,660	9.54	8,300	9.29	118,000	9.13
20	2,000	---	1,010	930	---	680	---	270	94	9.43	40,000	9.47	60,000	6.13	([a])	6.21
35	2,000	940	740	850	310	200	100	83	89	9.51	35,000	9.38	75,000	6.22	([a])	6.16
40	2,000	1,070	810	420	240	88	77	75	69	9.53	590	9.47	8,100	9.16	---	8.98
	pH 7.48															
0	3.8	---	4.2	3.5	---	2.0	---	2.7	2.0	7.74	2.6	7.59	3.7	7.63	3.8	7.64
7	3.8	---	1.5	3.9	---	2.1	---	2.0	4.1	7.70	9.0	7.65	3.7	7.69	12.0	7.31
16	3.8	---	3.9	3.8		1.1	---	4.5	5.7	7.69	17.0	7.64	121.0	8.50	400	6.95
20	3.8	---	4.8	3.7	---	6.9	---	8.0	35.0	7.70	134.0	7.44	510.0	6.41	670	6.14
35	3.8	3.3	5.7	5.3	6.6	8.1	8.5	7.7	130.0	7.45	760.0	6.49	260.0	6.00	900	[b] 7.86
40	3.8	4.5	6.2	5.8	4.7	7.2	11.5	10.0	51.0	7.66	310.0	6.59	360.0	5.91	97.0	5.67

[a] Countless.
[b] Stopper had blown out.

METHOD OF CLEANING

A study was made of the number of eggs that contained bacteria in each lot of the various experiments recorded in table 1. For this study, samples of the whites and of the yolks were plated on 0.5-percent glucose agar and the plates incubated 48 hours at 35° C. Since it was necessary to save the whites and yolks for other determinations, the bacteriological sampling technic was not ideal. And egg was not listed as infected unless the plate count was over 100 colonies per cubic centimeter. The percentage of eggs that contained bacteria in the whites and in the yolks are shown in table 7.

It is apparent that only a small percentage of the eggs that were clean before washing contained bacteria, regardless of the method used in washing them. Nearly all of the extremely dirty eggs contained bacteria in both whites and yolks, except the lot that was cleaned with sandpaper, in which some of the eggs were free from micro-organisms. In nearly every lot of naturally dirtied eggs some eggs contained bacteria, but the percentage of eggs having infected whites was no greater in the washed than in the unwashed eggs. The yolks were infected in a larger percentage of cases than the whites. One hundred percent of the yolks of the untreated naturally dirtied eggs contained bacteria; none of the other lots showed as many.

TABLE 7.—*Percentage of eggs listed in table 1 that contained bacteria in white or yolks after being cleaned by various methods and cleansing agents*

WHITES

Cleansing agent and treatment	Eggs washed and held at 35° C.				Eggs washed and held at room temperature, 20°–25° C., for 30 days		
	27 days, clean, in cartons	30 days			Clean	Extremely dirty	Naturally dirtied
		Clean	Extremely dirty	Naturally dirtied			
Water	0	0	100		0	60	
H_2SO_4 N/10	0	0	100		0	100	
NaOH N/10	0	0	100		0	100	
Water glass	0	0	100		0	100	
Water, oiled	0	0	100		0	100	
Oiled	0	0		33	0		0
Soap	9	8	100	0	8	100	25
Wyandotte powder	8	0	100		0	100	
Sapolio	0	0	100		0	100	
Hypochlorite			100	50		100	50
Sandpaper			50	50		33	20
Hypochlorite, oiled				33			67
Sandpaper, oiled				17			25
Soap, oiled				50			40
Dirty, unwashed			100	40		50	67
Clean, unwashed	0				0		
YOLKS							
Water	18	0	100		0	40	
H_2SO_4 N/10	8	0	100		0	100	
NaOH N/10	9	0	100		10	100	
Water glass	8	0	100		0	100	
Water, oiled	0	0	100		0	100	
Oiled	0	0		60	17		42
Soap	9	0	100	60	17	100	33
Wyandotte powder	8	0	100		20	100	
Sapolio	0	0	100		0	100	
Hypochlorite			100	75		100	67
Sandpaper			50	80		33	40
Hypochlorite, oiled				67			67
Sandpaper, oiled				58			25
Soap, oiled				50			50
Dirty, unwashed			100	100		83	100
Clean, unwashed	0				0		

Some of the solutions used in washing the eggs were selected because of their germicidal activity, so that the bacteria which were in the soiled material on the surface of the shell might be killed during the washing process. The hypochlorite solution was one of the most active germicides used. One dozen eggs that had been dipped in a manurial compost were washed in a 1:100 solution of the hypochlorite. As soon as the washing was completed, a sample of the dirty solution was plated on agar. At stated intervals thereafter, samples were plated for a bacterial count. The results of this test are shown in the following tabulation:

Time	Bacteria per cubic centimeter
At start	30,000
Half minute	25,600
1 minute	20,000
2 minutes	16,800
5 minutes	12,200
15 minutes	10,900
30 minutes	3,000
1 hour	2,500
2 hours	3,100
4 hours	1,800
8 hours	0

When the washing was completed, there were only 30,000 bacteria per cubic centimeter present, and before the end of 8 hours all of these were dead. It is probable that the vegetative cells were all destroyed by the time the washing was finished, and that the 30,000 bacteria represented only the more resistant spores.

This experiment shows the difficulty of destroying all of the bacteria in the water while the eggs are being washed.

In order to lessen the time required for the experiments the eggs were stored at 35° and at 22° to 25° C. To determine whether such experiments would reflect the results that would be obtained at a lower temperature, an experiment was made in which the eggs were stored for 5½ months at 2°. In this experiment naturally dirtied eggs were used. Four different treatments as shown in table 8 were studied.

TABLE 8.—*The growth of bacteria in 3 dozen naturally dirtied eggs receiving each treatment shown and then stored 5½ months at 2° C. and for 3 additional weeks at 25° and then examined for bacteria*

[One half the eggs were examined at the end of the 5½ months and the remainder after the 3 additional weeks]

Treatment	Average loss in weight	Eggs showing bacteria at end of 5½ months cold storage at 2° C	Eggs showing bacteria at end of 5½ months cold storage at 2° C followed by 3 weeks at 25°
Naturally dirtied eggs	*Percent*	*Percent*	*Percent*
Control, no treatment	----	11	[a] 41
Oil-dipped	0.7	0	11
Washed with Sapolio	3.7	[b] 20	[b] 27
Washed with Sapolio and oil-dipped	.9	[a] 0	[a] 12

[a] 17 eggs in the experiment.

[b] 15 eggs in the experiment.

Fewer dirty eggs developed bacterial infection at the low than at the two higher temperatures. However, attention should be called to the fact that the eggs used in this experiment were not an aliquot of those used to obtain the results for naturally dirtied eggs reported in table 7. Infection was less pronounced in the oil-dipped eggs than in those not oil-dipped. This finding cannot be accepted as general, however, for under some conditions infection seems to develop more readily in the oil-dipped eggs.

EFFECT OF WASHING ON DIFFERENT EGG CHARACTERS

Dry-Matter Content

Determinations were made of the percentage of dry matter in the whites and in the yolks of the individual eggs listed in table 1. The white loses water to the air by evaporation and to the yolk by osmosis. The oil-dipped eggs contained less dry matter in the white and more dry matter in the yolk than did the eggs which were not oil-dipped. This is in agreement with the previously demonstrated effect of oil-dipping on the pH values and the passage of water from the white to the yolk. No effect of washing on this change in dry-matter content was indicated.

Flattening of Yolk

As an egg ages, the yolk takes up progressively more and more water from the white, and when the egg is broken into a dish, the tendency of the yolk to flatten increases until in extreme cases the egg cannot be broken without the yolk membrane breaking. The flattened condition can be expressed in numerical units by dividing the height by the width. This factor obtained from eggs on the day they are laid is about 0.41. The application of this method has been described by Sharp and Powell (*28*). Such measurements were made on the yolks of the eggs of table 1, held at room temperature No difference in yolk quality between the washed and unwashed eggs as indicated by this ratio was noted. The value for the oil-dipped eggs was about 0.400, which is only slightly lower than that for fresh eggs. The value for the eggs receiving the other treatments was very low and many yolks broke. The difference between the oil-dipped eggs and the eggs receiving the other treatments was due to the retention of the carbon dioxide.

The extremely dirty eggs were so weak or so badly decomposed that it was possible to obtain measurements on a few yolks only The values for naturally dirtied eggs were somewhat lower than those for clean eggs. In this case, however, there was nothing in favor of the unwashed eggs over the cleaned eggs. The values were not affected by the cleaning before oiling treatment as compared with oiling without cleaning.

Hydrogen-Ion Concentration

A number of workers (*2*, *3*, *11*, *13*, *27*) have shown that the hydrogen-ion concentration of the egg decreases as it gets older, owing to a loss of carbon dioxide, the pH value of the white increasing from about 7.6 to 9.5 and that of the yolk from 6.0 to 6.8.

The pH values of the whites and yolks of the eggs referred to in table 1 was determined. No effect of the washing treatments could be demonstrated. Many of the eggs which were infected with bacteria showed abnormal pH values. The pH values of the oil-dipped eggs were much lower than those of the eggs that were not oil dipped.

Appearance

In appearance the eggs washed with Sapolio or Wyandotte powder were very much like clean, unwashed eggs; those cleaned with soap were decidedly shiny in appearance and slightly velvety to the touch. The eggs cleaned with acid or alkali had a mottled appearance; those dipped in oil were shiny.

Internally, the oil-dipped eggs were far superior to the others, and those that were washed before they were dipped were the equal of those that had not been washed before they were dipped. The mere washing of the eggs did not seem to cause them to break down at a more rapid rate.

DISCUSSION

This investigation indicates that with the solutions used, the washing of eggs caused no increase in rate of loss of weight as compared with that of unwashed eggs. It does show that the use of abrasive materials, such as sandpaper and sand, may cause a slight increase in the rate of evaporation.

The experiments here recorded show an approximately linear relationship between time and the evaporation of water from eggs at constant humidity. The loss in weight, so far as the writers have followed it did not yield a parabolic curve such as was obtained by Greenlee (*10*). It is obvious, however, that if enough water is permitted to evaporate from the eggs, the rate of loss in weight must eventually decrease Under conditions of commercial storage the parabolic form of the loss-in-weight curve might be obtained, for during the early period of storage, the fillers and cases absorb moisture from the eggs, whereas toward the end of the storage period the fillers and cases are more nearly in equilibrium with the humidity of the atmosphere, and consequently the loss in weight of the eggs is not so rapid.

The more recent investigations of Almquist and Holtz (*4*) have created some uncertainty as to the best method for determining porosity. The method recommended by them consists in immersing eggs in an alcoholic solution of methylene blue for 2 minutes, then removing the eggs and opening them. The porosity is indicated by the number of blue spots on the inside of the shell, and is expressed in terms of a set of standard shells showing varying degrees of staining. Almquist and Holtz found that loss in weight increased with porosity, as determined by their method, but they did not give the correlation coefficients. They state that the eggs were held at constant humidity, but they do not state what humidity was used. From the great loss in weight reported, they must have used a very low humidity, which may account for their results.

Almquist and Holtz have criticized the method used in the present experiments for determining pores, intimating that the changes in pressure would open the pores. This criticism is hardly justified by the facts. The number of pores was repeatedly determined on a series of eggs at weekly intervals for 6 weeks by the water-immersion vacuum method. While it is true that the number of pores increased or decreased slightly with some eggs, this change in number was usually within the possible error of counting, and, on the whole, the change in number was not nearly so great as the increase in porosity which Almquist and Holtz report as having occurred without any vacuum or pressure treatment at all.

This great increase in porosity with time, as determined by the Almquist and Holtz method, is rather puzzling, for if this increase in porosity is significant the eggs should lose weight at an increasing rate with time of holding. This does no appear to be the case. Minor factors, such as increase in osmotic pressure and air cell, could not counterbalance the effect, since the increase in porosity is often many times that of the freshly laid egg.

While the water-immersion vacuum method for determining pores leaves much to be desired, yet within fairly narrow limits, the method yields the same results whether the porosity is determined soon after the eggs are laid or after they have been stored. This agrees more nearly with the fairly constant loss in weight of the eggs under constant conditions. The pores which are shown on normal eggs are true channels through the shell as can be readily seen by examining them under a relatively low-power microscope. The significance of the water-immersion vacuum method may also be questioned because sanded eggs show a great increase in porosity and the increased rate of loss in weight is not in proportion.

Attention should be called to the importance of the alcohol in the Almquist and Holtz method. If water is used as a solvent, much the same porosity is indicated as is obtained with the water-immersion vacuum method; if alcohol is used as a solvent, the indicated porosity is much greater. A preliminary treatment of the eggs with alcohol does not increase the porosity as determined by the water-immersion method, however.

Stewart,[3] working in this laboratory, obtained about the same correlation between loss in weight and shell thickness as the writers obtained between porosity and loss in weight.

A relation between the number of pores and the strength of the shell, as indicated by the correlation coefficient +0.258 ± 0.033, was found. This indicates that up to a certain point the stronger the shell, the greater the number of pores. If, however, the shell is too porous the strength is lessened.

More extensive experiments have been in progress for some time in an effort to gain information on the factors involved in the loss in weight. While the evidence still points to the importance of the pores as one factor under some conditions the relation between the number of pores and the loss in weight is not as definite as was found in these experiments.

It seems reasonable to assume that bacteria and molds enter the egg by way of the large pores. Pennington[4] has shown that mold growth on the inside of an egg begins at the large pores. Since the large pores are relatively few in number, an egg might be soiled without the material actually covering a pore. Such eggs might escape infection. If the dirt happened to cover a portion of the shell containing a pore, the chance of infection would probably be greater. It has been assumed that the washing operation would spread the infected material over the surface of the eggs and increase the chances of some of it entering the pores. Jenkins, Hepburn, Swan, and Sherwood (*14*) have shown that washing dirty eggs increased the number that showed bacterial infection during storage. The data herein presented are too meager to warrant a definite conclusion on this point.

Rettger (*21*), Hadley and Caldwell (*12*), and others have demonstrated that very few clean newly laid eggs contain bacteria. The writers' observations confirmed this finding; very few such eggs showed infection even after being held at 35° C. for 30 days. On the other hand, eggs that had been heavily coated with manure and allowed to dry overnight contained bacteria after storage, regardless of the means of washing employed. A large number of the eggs that had been taken from the nest as "dirties" were found to contain bacteria after 30 days of storage. The washing of dirty eggs did not seem to increase greatly the number which were found to contain bacteria at the end of the storage period.

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[3] STEWART, G. F. SHELL CHARACTERISTICS AND WEIGHT LOSSES OF HEN'S EGGS. 1933. (Unpublished.)

[4] PENNINGTON, M. E. Private communication.

The humidity of the air in which the eggs are kept is a very important factor in determining whether dirty or washed dirty eggs will show the greatest spoilage.

These experiments indicate that washing naturally clean eggs with clean water and germicidal solutions has little or no effect upon the keeping quality of the eggs. This conclusion appears to be in opposition to the current belief that washing in itself causes eggs to deteriorate. Washed dirty eggs, however, do not keep so well as naturally clean eggs. The main reason for the deterioration of washed eggs commercially rests in the fact that the eggs were dirty before they were washed, and consequently the chance of bacterial infection was greatly increased. The number of washed or cleaned eggs which will show bacterial spoilage will depend upon the condition of the eggs before they were washed or cleaned and upon their treatment.

Some incidental observations on the effect of cleaning dirty eggs by abrasion indicate that this method does not insure against bacterial infection.

SUMMARY AND CONCLUSIONS

Eggs washed with a number of different solutions did not lose weight during storage at high temperatures more rapidly than unwashed eggs.

Oil-dipped eggs showed a smaller loss in weight during storage than eggs that were not oil-dipped.

The loss in weight was at a uniform rate for the length of the experimental period, 30 days.

A significant, positive correlation (+0.450 ± 0.057) between the number of pores in the eggshell and the loss in weight of the egg was found.

A small positive correlation (+0.258 ± 0.033) between the number of pores and the breaking strength of the shell was found.

Attention is called to the possibility that infection from bacteria may take place through the relatively few large pores of the shell.

The germicidal action of egg white having a high pH value is evidenced at temperatures from 0° to 40° C.

From the results here reported it is concluded that there is no foundation for the common belief that washing in itself causes eggs to deteriorate, if they are properly handled after washing. The deterioration of washed eggs is caused by bacterial infection of the egg from the dirt that was on the shell. The only effective remedy is to prevent the eggs from becoming dirty.

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A MORPHOLOGICAL STUDY OF BLIND AND FLOWERING ROSE SHOOTS, WITH SPECIAL REFERENCE TO FLOWER-BUD DIFFERENTIATION[1]

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INTRODUCTION

The formation of nonflowering shoots on forced rose plants represents a great economic loss to rose growers, for in certain varieties this flowerless growth may bring about a 50-percent reduction of each year's potential rose crop.[3]

It is well known that under greenhouse conditions the flowering rose shoot reaches maturity in approximately 45 days, whereas the blind shoot ceases growth after about 30 days and produces a new shoot from the last axillary bud. In the majority of cases this new shoot continues with blind growth for 30 days, at the end of which time it produces a new shoot in the same manner as its parent. The process of flower differentiation in the rose has not yet been established, nor has the author been able to find any report of an anatomical or morphological study of blind and normal rose wood. This paper reports the results of studies made on blind and flowering rose shoots of the variety Briarcliff.

REVIEW OF LITERATURE

The first important work concerning the causes of blindness in roses was done by Corbett.[4] He attributed the production of blind wood to inheritance. The relation of blind-wood production to length of day is being studied by Grove.[5] In a recent report by the present writer,[3] the following results were presented:

> A correlation between the physiological behavior and chemical differences of blind and flowering rose shoots indicates that blindness in the rose is a physiological rather than a genetic or pathological condition.
>
> A combination of pruning and budding experiments indicates that blindness is a result of the stock and is not due to impotency of the buds. This point is emphasized by the differences in the chemical composition of blind and flowering wood.
>
> Growth and differentiation were definitely affected by the monthly hours of illumination and the available nitrate supply. A decrease in illumination decreased both flower and blind-shoot production, while the normal increase in illumination in the spring months increased flower production more rapidly than blind-shoot production.
>
> With an increase in soil nitrates blind-shoot formation decreased and flower production increased; with a decrease in soil nitrates blind-shoot formation increased and flower production decreased.
>
> The chemical analyses indicate that blindness is associated with high percentages of noncolloidal nitrogen and insoluble carbohydrates, whereas the flowering shoots contain high percentages of reducing sugars.

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[1] Received for publication Aug 9, 1933; issued February 1934

[2] The author expresses his appreciation to Prof J. R Cooper and Dr. L M Turner for suggestions and criticisms throughout the progress of this work.

[3] Hubbell, D. S Unpublished thesis, Iowa State College. 1932.

[4] Corbett, L C IMPROVEMENT OF ROSES BY BUD SELECTION, OR BLIND VS FLOWERING WOOD FOR ROSE CUTTINGS. Mem. Hort. Soc. N.Y. 1: [93]-101, illus. 1902.

[5] Grove, L D. Unpublished material, Iowa State College.

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Since this work indicated that flower-bud formation is influenced by various cultural practices, a successful interpretation of these influences would depend upon a definite understanding of the time and extent of flower-bud differentiation. In order to obtain such an understanding it would be necessary to make a complete anatomical analysis of the two types of shoots. The purpose of the study here reported was to establish through morphological methods whether blindness in roses is a genetic or a physiological condition. When this point has been established the foundation for the control of blindness in roses will have been laid.

MATERIALS AND METHODS

Beginning December 1, 1932, 10 buds from flowering rose shoots and 10 buds from blind rose shoots were tagged each day until January 4, 1933. Each day the mature stems of blind and flowering stems were selected. The stems were cut back to the buds occupying the fourth axillary position and these buds were collected and studied after they had attained the desired age. The collection consisted of 350 buds and shoots from apparently blind wood and 350 normal buds and shoots. The buds and shoots collected ranged in age from 1 to 35 days.

Immediately after collection the buds and shoots were killed in formalin acetic alcohol. The material was then fixed, cleared, embedded, and sectioned according to the standard method as outlined by Chamberlain.[6] The slides were stained with safranine and light green.

RESULTS

DIFFERENTIATION AND DEVELOPMENT OF FLOWERING SHOOTS

Some differences were noted in the rate of shoot development among buds and shoots of the same age. In general, however, the majority of the buds developed at the same rate for the entire 35 days. Of the shoots and buds gathered, 3.8 percent developed blind wood, 2.8 percent failed to develop it, and 93.4 percent developed normally.

Figure 1 and plate 1 show the development of a normal flowering rose shoot from the purely vegetative bud to the complete formation of the floral parts. The first evidence of differentiation was found 8 days after the axillary bud had been made to assume the terminal position by the removal of the terminal bud (fig. 1, *D*). At this time a broadening and thickening of the floral axis was noted. The ninth day gave evidence of sepal protuberances pushing up from the sides of the bud (fig. 1, *E*). The first stages of petal primordia were observed after the lapse of 12 days (fig. 1, *G*). On December 30, when the bud was 20 days of age pistil primordia appeared as small protuberances on the bottom of the recepticale cup (fig. 1, *J*). At this stage the stamen primordia were also visible, although they were not clearly differentiated into anthers and filaments until the twenty-second day (fig. 1, *K*). By December 25 the stamens and pistils were so well differentiated that their component parts could be readily identified, and the petals had so far developed as to enclose the stamens and pistils (fig. 1, *L*).

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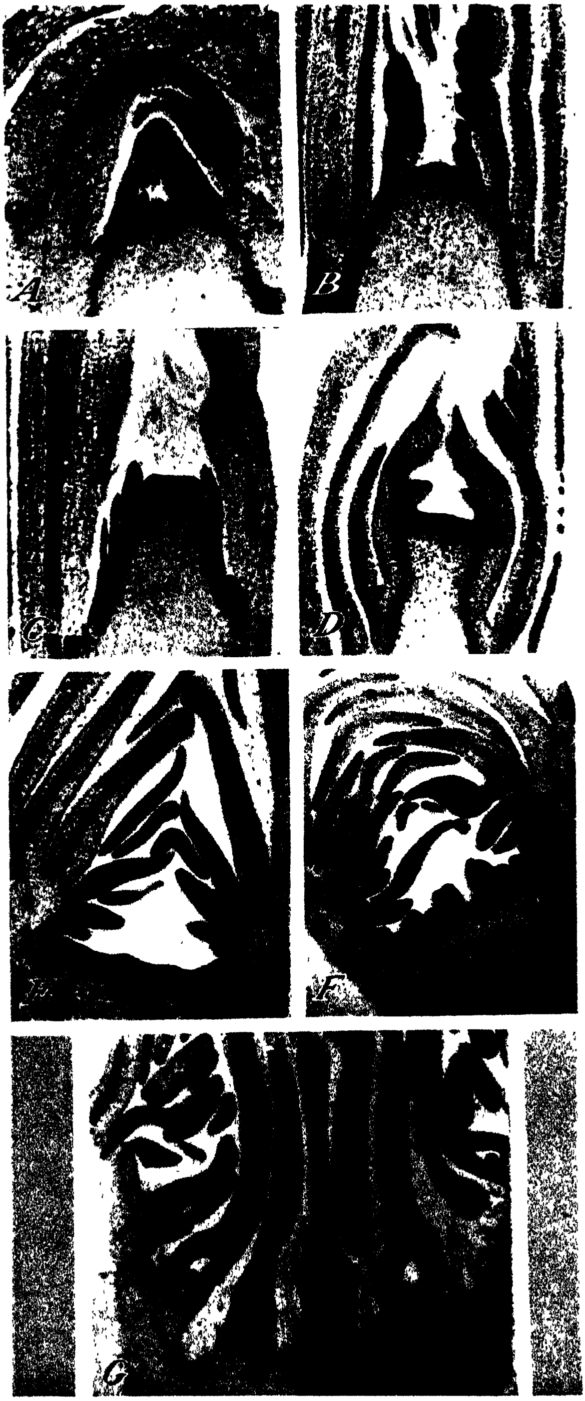
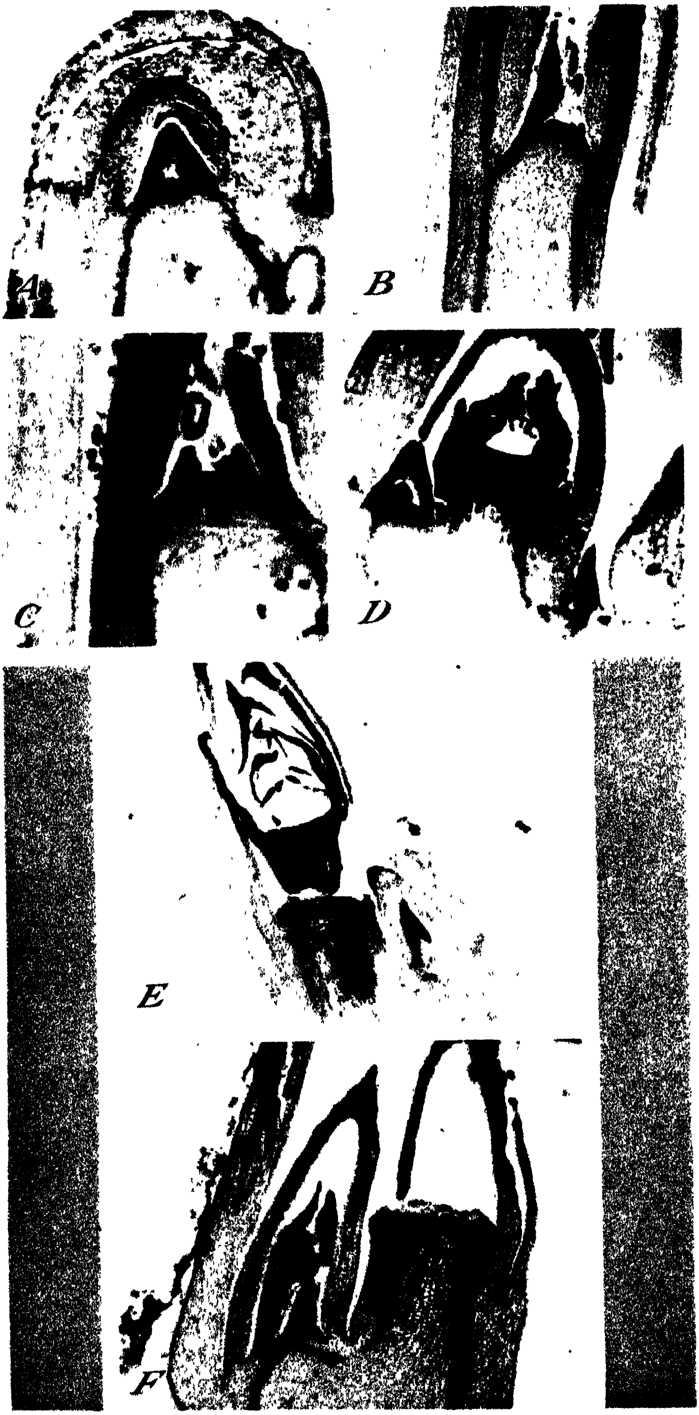
[6] CHAMBERLAIN, C. J METHODS IN PLANT HISTOLOGY. 3d rev. ed., 314 pp., illus. Chicago. 1915.



PHOTOMICROGRAPHS OF LONGITUDINAL SECTIONS THROUGH FLOWERING ROSEBUDS AT VARIOUS STAGES OF DEVELOPMENT

A, Immature bud 1 day old *B*, 8-day shoot showing the first stage of differentiation. *C*, 10-day shoot showing the formation of sepal primordia *D*, 12-day shoot with evidence of petal primordia. *E*, 21-day shoot showing pistil protuberances and stamen formation. *F*, 23-day shoot showing advanced stages of pistil primordia. *G*, 25-day shoot showing complete development of all floral organs × 80.



Photomicrographs of Longitudinal Sections Through Blind Rose-buds at Various Stages of Development.

A, Immature bud at the end of the first day. *B*, Signs of differentiation at the end of 10 days. *C*, Sluggish differentiation at 20 days *D*, Evidence of abortion at 28 days, with petal primordia just beginning to develop *E*, Evidence of abortion after complete petal formation. *F*, Axillary bud starting active growth after abortion of the terminal bud. × 80.

DEVELOPMENT OF BLIND SHOOTS

The development of the buds taken from blind stems was very irregular, as is shown in plate 2. Of the shoots and buds gathered, 5 percent formed flowers, 10 percent failed to develop beyond the axillary stage, and 85 percent formed blind shoots. The growth rate of the actively growing blind shoots was so lacking in uniformity that no definite dates of differentiation could be determined. Figure 2 shows sections of buds and shoots which displayed the greatest rate of maturity in each day's collection.

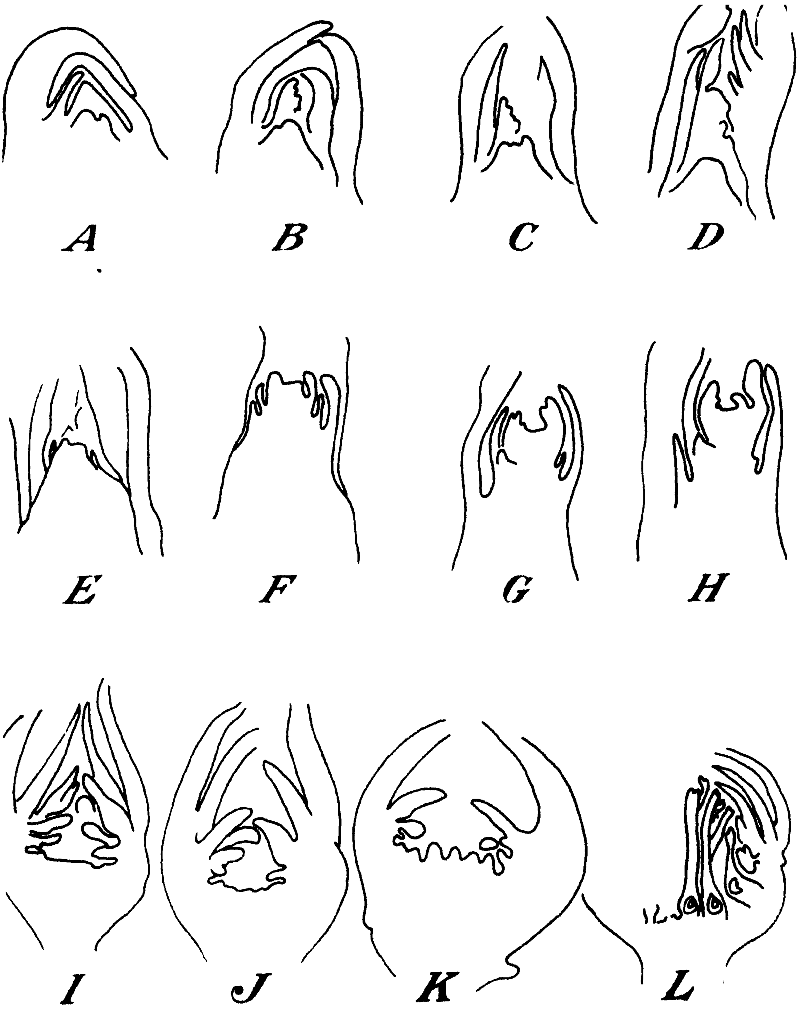


FIGURE 1.—Outline drawings of longitudianl sections through flowering rose-buds, showing the average stages of development from 1 to 25 days. *A*, 1 day, *B*, 3 days, *C*, 5 days, *D*, 8 days, *E*, 9 days, *F*, 10 days, *G*, 12 days, *H*, 14 days, *I*, 18 days, *J*, 20 days, *K*, 22 days, *L*, 25 days × 50

Figure 2, *C*, shows the characteristic flattening of the main axis which was the first indication of flower differentiation noted in the flowering shoots. This initial development took place 2 days later in the blind shoots than in the flowering shoots. At the end of the twenty-fourth day (fig. 2, *E*) the sepal primordia were very prominent, and definite petal primordia appeared from 2 to 6 days later, as is shown in figure 2, *F* and *G*. The formation of petals and sepals, together with a broadening of the receptacle cup, continued until the shoot was 34 days of age. At that time, without any evidence of pistil or stamen primordia, the bud showed clear signs of abortion (fig. 2, *I*). The condition of the blind shoot after complete abortion of the immature bud is shown in figure 2, *J*. The development of the new shoot, which normally begins growth from the last axillary bud, was in evidence at the end of 32 days, and at the end of 35 days the new shoot had assumed active growth (fig. 2, *I* and *J*).

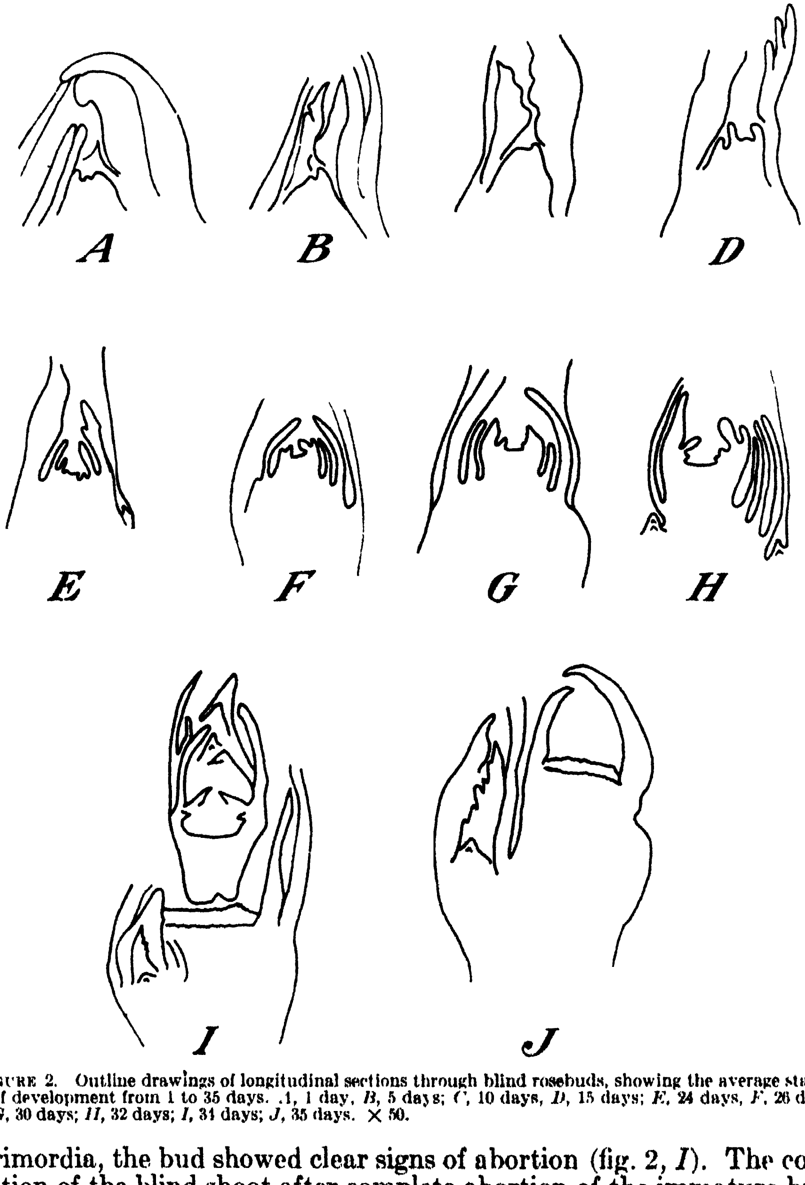


FIGURE 2. Outline drawings of longitudinal sections through blind rosebuds, showing the average stages of development from 1 to 35 days. *A*, 1 day, *B*, 5 days; *C*, 10 days, *D*, 15 days; *E*, 24 days, *F*, 26 days *G*, 30 days; *H*, 32 days; *I*, 34 days; *J*, 35 days. × 50.

SUMMARY AND CONCLUSIONS

A study was made of the growth and development of blind and flowering rose shoots with special reference to the date of flower-bud differentiation and the morphological differences between blind and flowering shoots. It was found that the approximate date of flower bud differentiation on flowering shoots was from 8 to 10 days after active growth had started. The complete formation of the flower with all parts completely differentiated was first noted at the end of the twenty-fifth day. Blind shoots were formed when the floral axis failed to develop a complete set of floral organs. Flower-bud differentiation started about 2 days later in the blind shoots than in the flowering shoots. The sepals and petals formed in the blind shoots, but no stamen or pistil primordia appeared. At the end of 30 days the undeveloped flower showed signs of abortion and 5 days later it had completely aborted. Signs of abortion were noted in a few cases at the end of 28 days.

Since the writer[7] has shown that blindness may be controlled by altering nutritional factors, and since abortion is associated with nutrition, it is evident that blindness is purelya physiological condition, in which an abortive bud is formed as a result of an improper balance of nutritional factors.

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[7] HUBBELL, D. S. See footnote 3.

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ZENILLIA LIBATRIX PANZER, A TACHINID PARASITE OF THE GYPSY MOTH AND THE BROWN-TAIL MOTH [1,2]

By PHILIP B. DOWDEN [3]

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INTRODUCTION

Early European rearings of the gypsy moth (*Porthetria dispar* L.) and the brown-tail moth (*Nygmia phaeorrhoea* Don.) for the purpose of obtaining natural enemies of these pests for liberation in the United States showed that *Zenillia libatrix* Panzer was a parasite of minor importance on both these insects. Later it was also reared in small numbers from the satin moth (*Stilpnotia salicis* L.). In 1928 a fairly large number of *Z. libatrix* were recovered from *P. dispar* reared at Vecs, Hungary, and study on the biology of the species was begun at the sublaboratory of the Bureau of Entomology, United States Department of Agriculture, in Budapest. This paper gives the results of the study and briefly discusses the attempts that have been made to establish the parasite in New England and the probability of its establishment.

REVIEW OF LITERATURE

References to *Zenillia libatrix* are fairly common. In 1908 Townsend (*17*)[4] briefly noted its habit of leaf oviposition and the fact that it is double-brooded. Pantel (*11, pp. 46, 48*) in 1910 placed it (under the genus *Myxexorista*) in his group II of leaf-ovipositing tachinids and figured the egg and an ovariole. Howard and Fiske (*8, pp. 90–91*) in 1911 and Burgess and Crossman (*5, p. 115*) in 1929 recorded it as a parasite of both the gypsy moth and the brown-tail moth and noted the number of flies liberated in the United States. In 1920 Baer (*1, p. 153*) gave a list of lepidopterous hosts attacked by *Z. libatrix*. Eidmann (*7*) in 1926 mentioned the fact that it is very polyphagous and its mode of gaining entrance into the host. Finally, in 1931, Brown (*4*) recorded it as a parasite of the satin moth and briefly indicated its life cycle.

DISTRIBUTION AND HOST RELATIONSHIPS

Zenillia libatrix is common throughout Europe. Rearings of gypsy and brown-tail moth material in connection with the work of the United States Bureau of Entomology show recoveries from

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[1] Received for publication Oct. 2, 1933, issued March, 1934.

[2] This study was conducted under the direction of C. W. Collins, in charge of the Bureau of Entomology field laboratory, Melrose Highlands, Mass., at its European sublaboratory at Budapest, Hungary, during 1929, 1930, and 1931, and at Melrose Highlands in 1932

[3] The writer expresses his gratitude to R. T. Webber for the use of his notes on *Zenillia libatrix* and for many helpful suggestions; to D. W. Farquhar for laboratory assistance at Melrose Highlands, to Ferencz Mihalyi for laboratory assistance at Budapest; and to W. F. Sellers, W E. Ripper, R C Brown, and Josef Ujhelyi for help in making collections in Europe.

[4] Reference is made by number (italic) to Literature Cited, p. 113

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Spain, France, Germany, Austria, Czechoslovakia, Hungary, Italy, Yugoslavia, and Russia. Wainwright (*18*) records it from England, and Lundbeck (*9*) from Denmark, Sweden, and Finland. Howard and Fiske (*8*, *p. 302*), Baer (*1*, *p. 153*), and Brown (*4*) state that the species is principally a southern form, but the largest collections made of it in gypsy-moth work were in Hungary, while in rather extensive rearings in Spain, Portugal, and Morocco only a single specimen (from Spain) was reared.

Zenillia libatrix is very polyphagous. A list of hosts recorded in European literature follows. Those starred (*) are species from which, during this study, the parasite has also been reared from field-collected material.

LEPIDOPTEROUS HOSTS OF ZENILLIA LIBATRIX RECORDED IN EUROPEAN LITERATURE AND REARED FROM FIELD-COLLECTED MATERIAL IN HUNGARY

Abrostola asclepiadis Schiff. (*1*, *3*, *8*, *9*)
Acronicta auricoma Fab. (*9*)
Brephos nothum Hbn. (*1*, *3*, *8*, *9*)
Bupalus piniarius L. (*1*, *7*, *9*)
Dasychira pudibunda L. (*1*, *3*, *8*, *9*)
Drepana cultraria Fab. (*18*)
**Euproctis chrysorrhoea* L. (= *Nygmia phaeorrhoea* Don.) (*1*, *9*)
Larentia autumnalis Strom. (*1*, *3*, *8*, *9*)
Liparis monacha L. (*2*)
Loxostege sticticalis L. (*10*)
**Lymantria* (=*Porthetria*) *dispar* L. (*1*, *3*, *8*, *9*)
Malacosoma neustria L. (*1*, *3*, *8*, *9*)
**Oxycesta geographica* Fab.[5]
Phlyctaenodes verticalis L. (*1*, *9*)
Porthesia similis Fuess. (*1*, *9*)
Pygaera anachoreta F. (*1*, *9*)
**Pygaera pigra* Hufn. (*1*, *3*, *8*, *9*)
Salebria marmorata Alph. (*13*)
**Stilpnotia salicis* L. (*4*)
Sylepta ruralis Sc. (*1*, *9*)
Thaumetopoea processionea L. (*1*, *3*, *8*, *9*)
Yponomeuta cognatella Hbn. (*9*)
Yponomeuta evonymella L. (*1*, *3*, *8*, *9*)
Yponomeuta padella L. (*1*, *3*, *8*, *9*)
Yponomeuta rorella Hbn. (*1*, *9*)

Although *Pygaera pigra* was the only field-collected host in which *Zenillia libatrix* overwintered, the species also passed the winter successfully in *Oxycesta geographica*, *Calocasia coryli* L., and *Acronycta rumicis* L. attacked at the Budapest laboratory.

In the United States at the Melrose Highlands (Mass.) laboratory, D. W. Farquhar and R. M. Seeley, under the direction of R. T. Webber, successfully used the silkworm (*Bombyx mori* L.) to rear a summer generation of the flies, and they had a large number of native lepidopterous larvae, representing 23 species, attacked by *Zenillia libatrix* in an attempt to determine whether there was a suitable alternate host for the species in New England. They found that the parasite was capable of completing a summer generation in at least two species, *Euchaetias egle* Drury and *Melalopha inclusa* Hbn., and the writer, on returning to Melrose Highlands, successfully brought it through the winter on *M. inclusa*.

ECONOMIC IMPORTANCE

From 1906 to 1910 only 177 adults of *Zenillia libatrix* were liberated in New England. They were obtained from brown-tail-moth caterpillars collected in Europe. In 1923 the Bureau of Entomology resumed the importation of enemies of the gypsy moth and the brown-tail moth from Europe, but since that time no heavy infestations of *Nygmia phaeorrhoea* have been investigated there and collections of these two species have been confined almost entirely to *Porthetria dispar*. Recoveries of *Z. libatrix* were negligible until 1927, when 4 puparia were reared from the gypsy moth at Moscenica, Yugoslavia. In 1928, 1,690 puparia were obtained from 230,000 *P. dispar* collected at Vecs, Hungary. Since that time recoveries have been small, exceeding 100 only twice; 163 were reared from 330,000 *P. dispar* collected at Galgamacsa, Hungary, in 1931, and 109 were reared from 320,000 *P. dispar* collected at Jánk, Hungary, in 1930.

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[5] Not recorded in literature but reared during this study.

Collections of satin-moth larvae were made in Vienna, Austria, from 1928 to 1930. In 1928, 40 *Zenillia libatrix* were recovered from 50,000 *Stilpnotia salicis;* in 1929, 124 were recovered from 90,000 larvae; and in 1930, 26 were reared from 90,000 larvae.

In 1930 about 6,000 brown-tail-moth larvae were collected in Hungary, but no *Zenillia libatrix* were reared. In 1931 no puparia of this species were reared from 1,500 *Nygmia phaeorrhoea* collected near Budapest, Hungary, but 22 puparia were reared from about 1,000 larvae collected at Oberpullendorf, Austria. In 1932, 186 *Z. libatrix* were reared from 5,600 larvae collected at the same place.

This summary shows rather conclusively that *Zenillia libatrix* is usually of slight economic importance as a parasite of the gypsy moth, the brown-tail moth, or the satin moth. Nevertheless, the fact that a large number of puparia were recovered at Vecs, Hungary, in 1928 indicates that the species may become important under favorable conditions. At times it may also be a valuable parasite of other host species which it attacks. Pustovoit (*13*) has recorded a parasitization by *Z. libatrix* of 10.5 percent on the procession moth (*Thaumetopoea processionea* L.).

TECHNICAL DESCRIPTION

ADULT

The adult fly was originally described as *Musca libatrix* by Panzer (*12*) in 1798. The original Latin description is very brief, but Lundbeck (*9, pt. 7, pp. 338 339*) has written the excellent redescription and synonymy which follow. Lundbeck calls the visible segments 2–5; the present writer calls them 1–4. Lundbeck omits the last segment entirely (his fifth, the writer's fourth).

1. *Z. libatrix* Panz.

1798. Panz. Faun. Germ. LIV, 12 (*Musca*)—1824. Meig. Syst. Beschr. IV, 400, 281 (*Tachina*) et 1838. VII, 256, 46 (*Exorista*)—1844. Zett. Dipt. Scand. III, 1163, 164 (*Tachina*)—1862. Schin. F. A. I, 464 (*Exorista*)—1891. B. B. Denkschr. Akad. Wiss. Wien, LVIII, 333 (*Myxexorista*)—1907. Kat. paläarkt. Dipt. III, 278—1907. Villen. Wien. Ent. Zeitg. XXVI, 254, 42—1921. Baer, Zeitschr. f. angew. Ent. VII, 153—1924. Stein, Arch. f. Naturgesch. 90, 6, 77, 14 (*Exorista*)—*Tachina fauna* Meig. 1824. l c. IV, 393, 268 et 1830. VI, 368 et 1838. VII, 256, 34 (*Exorista*)—1862. Schin. F. A. I, 464 (*Exorista*)—1900. Villen. Bull. Soc. Ent. de Fr. 159, 11, 12 et 1907. Wien. Ent. Zeitg. XXVI, 248, 5—1907. Kat. paläarkt. Dipt. III, 278.

All brownish-yellow pruinose species. Male. Frons above a little narrower than the eye, somewhat protruding. Orbits much broader than frontal stripe, yellow or almost golden; cheeks yellow above, silvery whitish below; jowls grey, nearly one third of the height of the eye; frontal stripe narrow, widening downwards, velvet black or brownish black. Weak outer vertical bristles present. Frontal bristles descending fully to the end of second antennal joint, two uppermost reclinate. Vibrissae ascending fully to the middle, the upper ones small. Orbits and jowls with black hairs. Occiput yellowish grey, with yellowish hairs, and black hairs behind postocular bristles. Eyes pale-hairy. Antennae black, third joint about four times as long as second; arista as long or fully as long as antennae, thickened in basal half. Palpi yellow. Thorax yellow pruinose, with four narrow black stripes, the median abbreviated behind, the lateral interrupted at the suture into two elongated spots; scutellum bright yellow. Thorax black-haired. Three sternopleural bristles. Abdomen quite yellowish or brownish yellow pruinose, basal segment a little darker; it is black-haired, with a pair of marginal bristles on second segment, a pair of discal and marginal on third and a pair of discal and a row of marginal on fourth segment. Legs black, a little greyish pruinose. Wings yellow at base and anterior margin, outwards slightly tinged; veins brown; first posterior cell narrowly open, ending near apex of wing; discal angle obtuse; apical cross-vein about straight. Squamulae yellow or deep yellow. Halteres yellow.

Female. Similar; frons a little broader, as broad as the eye.

Length 6.5–8 mm.

* * * * * * *

Remarks: The species is known to vary in colour, being sometimes greyish, but our specimens are all brownish yellow. On account of its varying it has been described under several names; to the above synonymy still can be added. *Tachina dolosa* Meig., *Exorista ancilla* Meig., *Myxexorista macrops* B. B., *M. grisella* B. B., *Zenillia perplexa* Pand., *Z. discerpta* Pand., *Z fulva* Pand., of these *fauna* Meig., *ancilla* Meig., and *grisella* B. B. belong to the greyish variety.

IMMATURE STAGES

Egg

The egg (fig. 1, *A*) is microtype, about 0.15 mm wide and 0.20 mm long. It is ovoid in shape, and there is a distinct micropyle at the cephalic extremity The thick upper surface of the chorion is rounded and heavily pigmented with conspicuous reticulations; the lower surface is thin, flat, and transparent. Beneath the chorion there is a thin but strong vitelline membrane enclosing the embryo. A thin layer of gelatinous material sticks the egg to the foliage and may be seen protruding slightly around the ventral margin of the egg.

First-Instar Larva

The larva (fig. 1, *B*) increases greatly in size during the first instar A specimen removed from the egg is 0.23 mm long and 0.10 mm wide The 5-day-old specimen figured is 0.45 mm long and 0.16 mm wide. Maximum growth attained in this instar is about 2 mm in length and 0.56 mm in width. The larva is cylindrical, tapering anteriorly and rounded posteriorly. It is composed of the pseudocephalon, bearing the antennal and sensorial organs, and 11 body segments The pseudocepnalon is unarmed, but on all the other segments except the eleventh the colorless cuticle bears rows of minute spines. The first three anterior (thoracic) segments are completely encircled with spines anteriorly On the fourth (first abdominal) segment the band of spines is broken in the pleural region. The fifth, sixth, and seventh segments have small groups of spines on their anterior margins in the ventral region. The eighth, ninth, and tenth segments have small anterior groups and also small posterior groups in the ventral region The eleventh segment is unarmed. The arrangement of the spines in rows and their comparative sizes are indicated in the figure. Those on the anterior margins of the segments are directed backward, those on the posterior margins are directed forward. In a small, young larva the spines are very conspicuous, but as the larva grows they become widespread and less prominent.

The buccopharyngeal armature of the freshly emerged larva is shown in figure 1, *D*. It has no articulations. The median tooth is well developed and the basal lobes are very lightly pigmented. There is a pair of lateral plates at the anterior end of the median tooth, and a single salivary-gland plate ventrad of the basal region. When the salivary-gland plate is turned on its side, a pair of minute openings can be seen at the anterior extremity. In fully developed first-instar larvae the buccopharyngeal armature presents a different appearance on account of the further sclerotization of the basal lobes (fig. 1, *E*).

The larva is metapneustic. The two posterior spiracles open on the dorsopleural portion of the last abdominal segment. The spiracular chambers (fig. 1, *C*) are about twice as long as broad.

The only sensory organs observed are the minute antennal and maxillary organs on each side of the mouth opening. The antennal organs are circular in outline

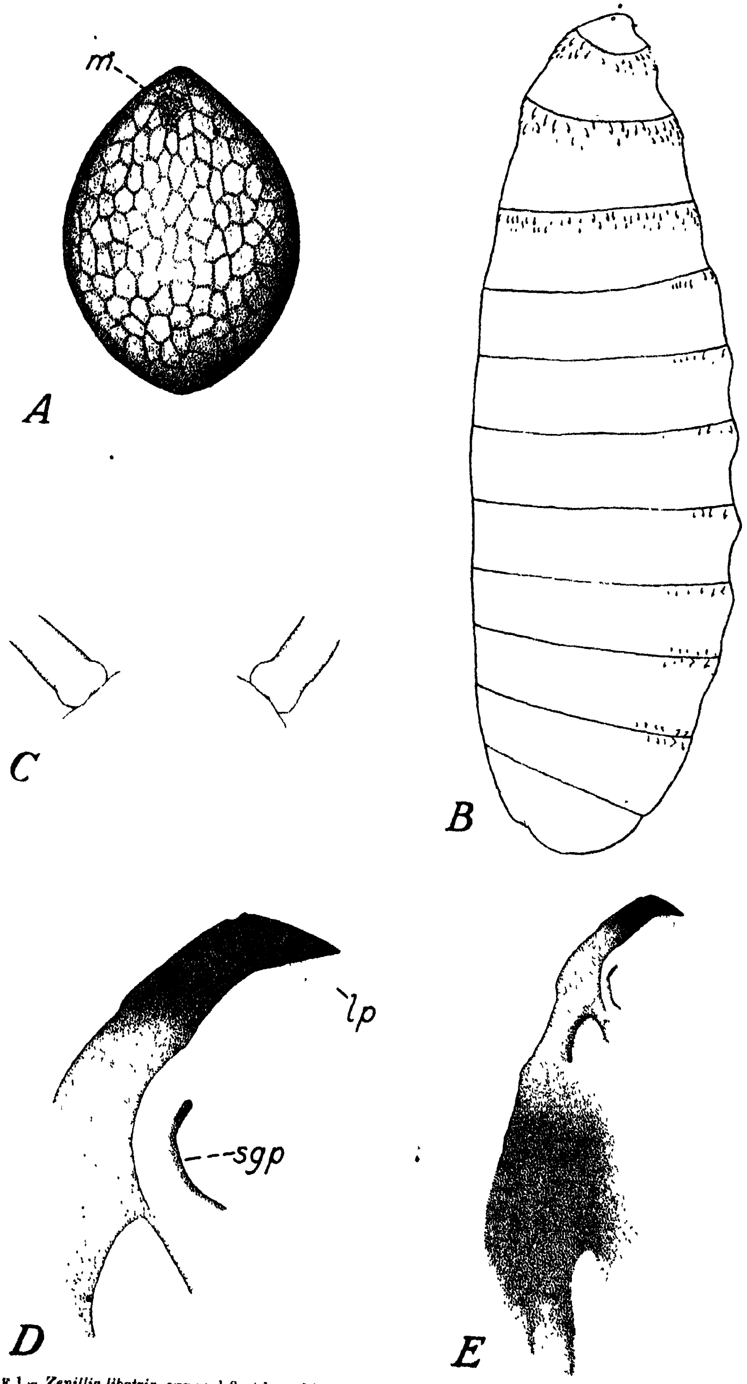

FIGURE 1.—*Zenillia libatrix*, egg and first larval instar: *A*, Egg, dorsal view, showing micropyle, *m* (× 220), *B*, larva, lateral aspect (× 250); *C*, posterior spiracular chambers (× 1,300); *D*, buccopharyngeal armature of freshly emerged larva, showing lateral plates, *lp*, and salivary-gland plate, *sgp* (× 1,000); *E*, buccopharyngeal armature of fully developed first-instar larva (×320).

and convexly protuberant, measuring 0.0025 mm in both height and breadth. Just below them are the maxillary organs, each composed of a group of minute points raised very slightly above the surface of the cuticle. Each group has 2 rather prominent points and above them 2 and below them 4 or 5 tiny points.

SECOND-INSTAR LARVA

The second-instar larva (fig. 2, *A*) is about 3.3 mm long and 0.9 mm wide when first formed. A larva just about to molt to the third instar was found to be 6.25 mm long and 2 mm wide. It is more robust than the first-instar larva, and the posterior extremity is truncate rather than rounded. The cuticle is still colorless. Although the cuticular spines are much more numerous than in the first instar, they are smaller and weakly pigmented, and therefore far less conspicuous. The pseudocephalon is unarmed. There are completely encircling bands of spines on the anterior margins of the first three segments. Segments 4 to 10, inclusive, have only very small groups of spines on their anterior margins, and these are all in the ventral region. The posterior margins of these segments, on the contrary, are well armed. Segments 4 and 5 are not completely encircled, for there are no spines in the dorsal region. Segments 6 to 10, however, have completely encircling bands on their posterior margins. Segment 11 has no spines on the anterior margin, but on the posterior margin there are from 15 to 20 rows. Below the spiracles of this segment there are several rows of spines directed away from the spiracles in a dorsoventral direction. The other spines are directed anteriorly. Figure 2, *A*, shows the arrangement of the spines, but there is considerable variation in the number of rows in different specimens. The spines on the anterior margins of the segments are pointed backward and those on the posterior border are pointed forward, as in the first instar.

In this instar the buccopharyngeal armature (fig. 2, *B*) terminates in a pair of lateral hooks. There are no articulations. The dorsal wing of the ventral plate is much longer than the ventral wing, but there is no progressive sclerotization of the basal wings as in the first instar. At the base of the lateral hooks there is a small sclerite, the hypopharyngeal plate, and dorsad of it the epipharyngeal plate. These sclerites are essentially the same in the second and third instars. They will be described in connection with the description of the third-instar larva.

The second-instar larva is amphipneustic. The anterior pair of spiracles (fig. 2, *C*) present two minute papillae, which open on the posterior border of the first body segment. There are no pigmented spiracular chambers connecting them with the tracheae, although the faint suggestion of an unpigmented tube may be found in prepared specimens. The posterior pair of spiracles (fig. 2, *D*) are well developed. Each has two papillae and often one of them is branched, as in the figure. The heavy spiracular chambers are about one and one half times as long as broad. The scar formed from the first-instar spiracles is prominent at the base of the spiracular openings.

The sensorial organs on the pseudocephalon are practically the same as in the first instar. The only difference is that they are raised a little higher. In this instar 4 pairs of small sensorial organs, having the form of fingerlike projections, were noted on the last abdominal segment, 2 pairs above the spiracles and 2 below.

THIRD-INSTAR LARVA

The third-instar larva (fig. 3, *A*) has the same form as the preceding instar. It is much larger, measuring 7.5 mm long and 2.5 mm wide to 11 mm long and 3.5 mm wide. The cuticle is colorless and transparent and armed as before with tiny spines, but in this instar the spines are relatively so small that it is hard to see them. The pseudocephalon is armed, bearing 3 or 4 short rows of spines on each side of the mouth opening. Segments 1 to 9, inclusive, have completely encircling bands of spines on their anterior margins. On segment 10 this band is incomplete, there being no spines in the dorsal region. Segment 11 has no spines on its anterior margin. The anterior bands on the first three segments are of nearly uniform width throughout, but the bands on the abdominal segments are much wider on the venter where they cover the pseudopodia. Proceeding posteriorly there is a diminution of spines on the dorsal region on the anterior margins of the segments. The first three segments have no spines on their posterior borders. Segment 4 has a few, and the number on each segment increases proceeding posteriorly. On the posterior margins of segments 7 to 11 there are completely encircling bands of spines. On segment 11 there may be as many as 20 rows directed anteriorly, and several rows below the spiracles have a dorsoventral

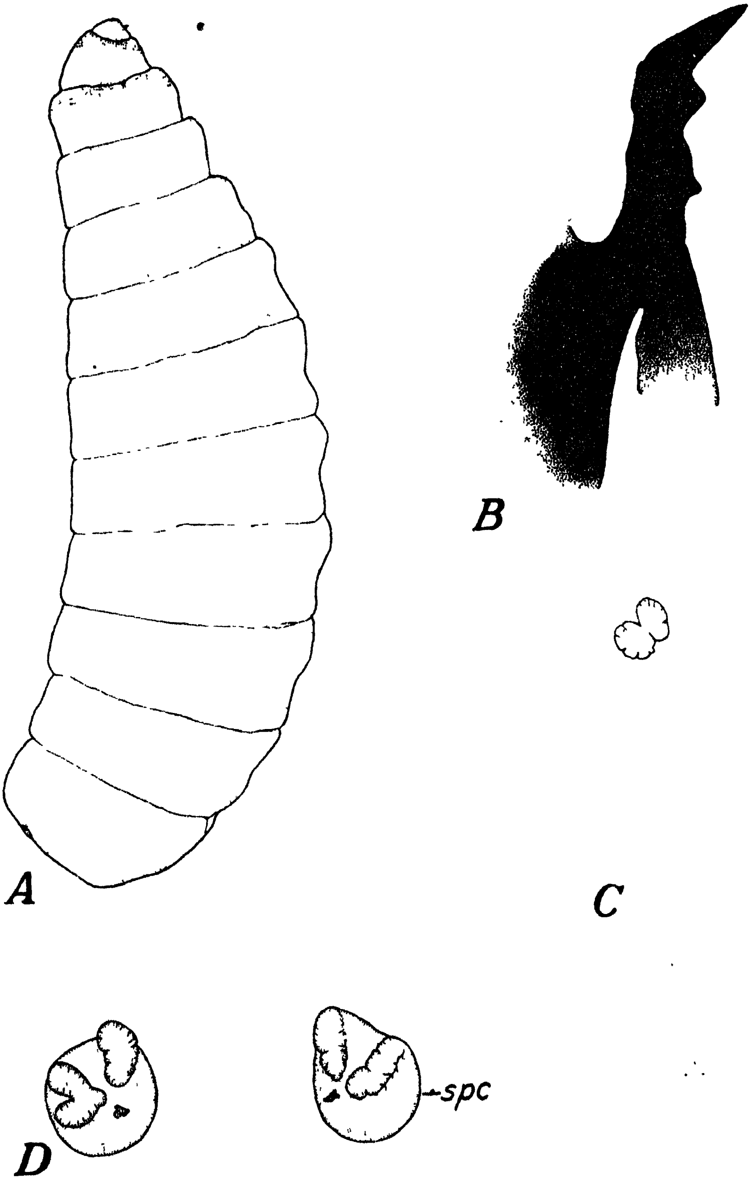

FIGURE 2.—*Zenillia libatrix*, second larval instar; *A*, Larva, lateral aspect (× 21); *B*, buccopharyngeal armature (× 160); *C*, anterior spiracles (× 600); *D*, posterior spiracles, showing spiracular chambers, *spc* (× 200).

direction. Figure 3, *A*, shows the disposition of the spines, but they are actually by no means as prominent as here indicated. The spines on the anterior margins of the segments point backward, and those on the posterior margins point forward as before.

The buccopharyngeal armature (fig. 3, *B*) is quite different from that of the second-instar larva. The lateral hooks are much straighter, there is an articulation between the intermediate region and the basal region, the dorsoanterior angle of the dorsal wing of the basal plate is strongly produced anteriorly, and there is a weakly sclerotized connection between it and the lower portion of the lateral hook. In many specimens a small opening is seen in the lateral hooks at the base of the ventral projection. It is believed that this is formed by the cells which generate the anterior hooks, as shown by Thompson (*16, p. 39, pl. IX, fig. 23*) in the larva of *Miltogramma punctatum* Meig., for in freshly molted third-instar larvae this opening is quite large. In many specimens the ventral projection of the lateral hooks seems to have a well-developed spine at its apex. This spine, though, is actually attached to the cuticle. Just above the intermediate region between the lateral hooks, the hypopharyngeal plate (fig. 3, *E*) may be distinguished. This is a small, irregularly shaped sclerite having two unsclerotized areas in the center of which are minute sensoria. The hypopharyngeal plate is attached basally to the intermediate region and anteriorly to the labium. The labium is a tough membrane armed with a varying number of well-developed spines. Just dorsad of the hypopharyngeal plate is the minute epipharyngeal plate (fig. 3, *F*). This plate varies considerably in shape. Often it is deeply emarginate. It has several unsclerotized areas bearing sensoria, as indicated in the figure.

The larva is amphipneustic in the third instar. The anterior spiracles (fig. 3, *C*) are well developed; they usually bear two papillae, and one specimen has been observed with three. The spiracular chamber is broad, and it widens abruptly into a cap fitting over the trachea. The posterior spiracles (fig. 3, *D*) are large, heavily sclerotized, and strongly raised. They are almost half as high as broad. Each spiracular plate has three respiratory slits. The molting scar is rather inconspicuous. The specimen figured was taken from a fairly young third-instar larva in order to show the respiratory slits well, but in an older larva almost the entire surface of the spiracle, with the exception of the slits and a small area around the molting scar, is pigmented to a deep black color.

The sensory organs on the pseudocephalon, antennal and maxillary, as well as the four pairs of fingerlike sensory organs on the eleventh segment, are the same as in the second instar, but they are even more prominently raised from the surrounding cuticle. No other sensory organs have been distinguished.

THE PUPARIUM

The puparia average about 8 mm long and 3 mm wide at the center. They are dull red. Both the anterior and posterior ends of the puparium are rounded. The posterior spiracles are situated a little above the longitudinal axis and just anterior to the apex. The spiracles are shiny black and raised almost half as high as their width. The anal opening is on the under side on the anterior margin of the eleventh segment. The anterior spiracles are very small but distinct.

The pupal respiratory apparatus (fig. 3, *G*) is of the reduced type, the prothoracic cornicles being absent. The tubes that usually connect them with the internal spiracles are present, and there is a small, slightly roughened, yellowish area in the cuticle of the prepupa where the apex of this tube comes in contact with the puparial shell. The internal spiracles are well developed. They bear about 200 respiratory papillae arranged along 5 or 6 radiating branches.

BIOLOGY AND HABITS

SEASONAL HISTORY

Zenillia libatrix passes the winter as a first-instar or second-instar larva within the host pupa and completes its development early in the spring. In the laboratory the first flies appeared April 6, 1928, May 18, 1929, and May 16, 1931. An adult male was taken in the field at Vecs, Hungary, on May 18, 1929.

The first adults of *Zenillia libatrix*, therefore, attack *Porthetri* *dispar* or some other host larva from about the middle of May until

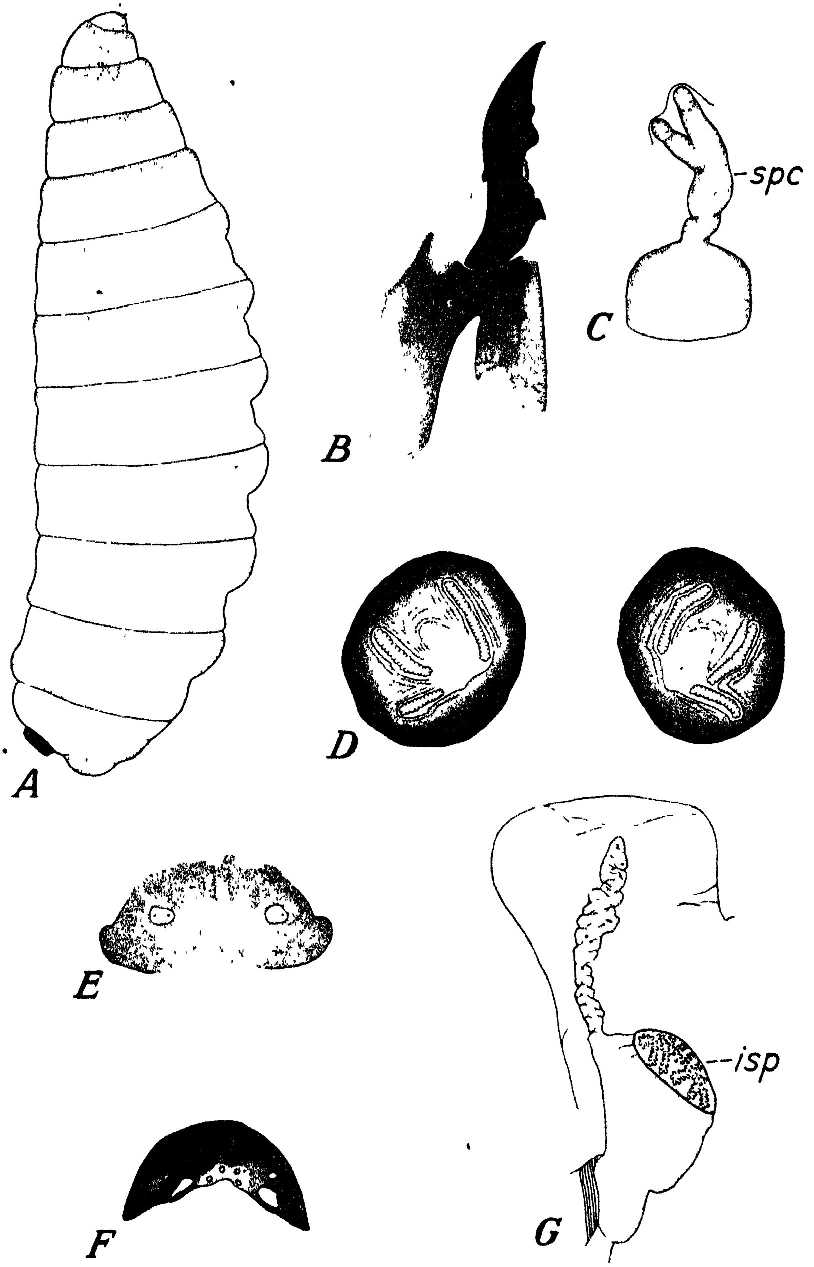

FIGURE 3.—*Zenillia libatrix*, third larval instar and pupa: *A*, Larva, lateral aspect (× 13), *B*, buccopharyngeal armature (× 75); *C*, anterior spiracles, showing spiracular chambers, *spc* (× 160), *D*, posterior spiracles, dorsal view (× 60); *E*, hypopharyngeal plate (× 425), *F*, epipharyngeal plate (× 500), *G* pupal respiratory apparatus, showing internal spiracle, *isp* (× 66)

the first of June or later. Puparia of *Z. libatrix* were recovered from field-collected *P. dispar* between June 22 and July 20 in Hungary. Adults issued between July 6 and August 6 in the laboratory, but probably somewhat later in the cooler forest. It is questionable whether another complete generation of *Z. libatrix* develops in nature. There is probably time, for a second summer generation is easily reared in the laboratory. Nevertheless, it seems doubtful, because of the scarcity of host material late in July and early in August and because flies issuing at that time could live long enough to attack larvae in which their progeny would overwinter. One female of *Z. libatrix* was taken at Vecs on August 25, 1928. The only field-collected larvae in which this parasite overwintered were *Pygaera pigra* Hufn. collected on October 6 and 10 near Budapest, Hungary.

In Hungary, therefore, *Zenillia libatrix* has at least two generations a year and possibly a partial third, while farther south it may have three regularly.

LENGTH OF LIFE

Zenillia libatrix adults live a considerable time under laboratory conditions. As many as five flies were held in glass-covered wooden boxes 5.9 by 7.9 by 3.9 inches (15 by 20 by 10 cm). They were fed lump sugar and honey solution (1 part of honey to 5 parts of water) held on sponges. When the flies were not being used, they were kept in a dark, cool place.

The average length of life of 18 mated females was 49.7 days, with a minimum of 27 and a maximum of 65 days; 21 males averaged 29.4 days, with a minimum of 8 and a maximum of 49 days; and 6 unmated females averaged 47.1 days, with a minimum of 34 and a maximum of 56 days. Of 4 males and 2 females issuing on July 12 which were given neither food nor water, 2 of the males lived 4 days, the other 2 males lived 5 days, and both females lived 5 days.

MATING

Zenillia libatrix adults mate readily in cloth-covered cages held in the light, but not in the direct sunlight. They prefer the morning hours. Temperatures from 64.4° to 77° F. (18° to 25° C.) are favorable. The male flies are particularly attracted by flying females. The average time in coitus, for 16 pairs, was 85 minutes, with a minimum of 35 and a maximum of 130. Although females up to 3 or 4 days old mate fairly readily, freshly emerged females and males from 2 to several days old mate best.

Female flies never mated more than once, but experimentation indicated that 1 male can fertilize about 8 females. One hundred eggs were examined from each of 8 females fertilized by a single male, and at least 93 percent of the eggs from each female were fertile. A similar examination of the eggs of 8 females fertilized by another male showed that the eggs of each of the first 7 females were at least 92 percent fertile, while the eighth female had only 3 percent of her eggs fertilized.

PREOVIPOSITION PERIOD AND REPRODUCTIVE ORGANS

The reproductive organs of a freshly emerged *Zenillia libatrix* female are shown in figure 4, *A*. Each ovary consists of a number of ovarioles (fig. 4, *B*). Dissections showed that in some small flies there were only 61, while in one very large fly there were 92 in one

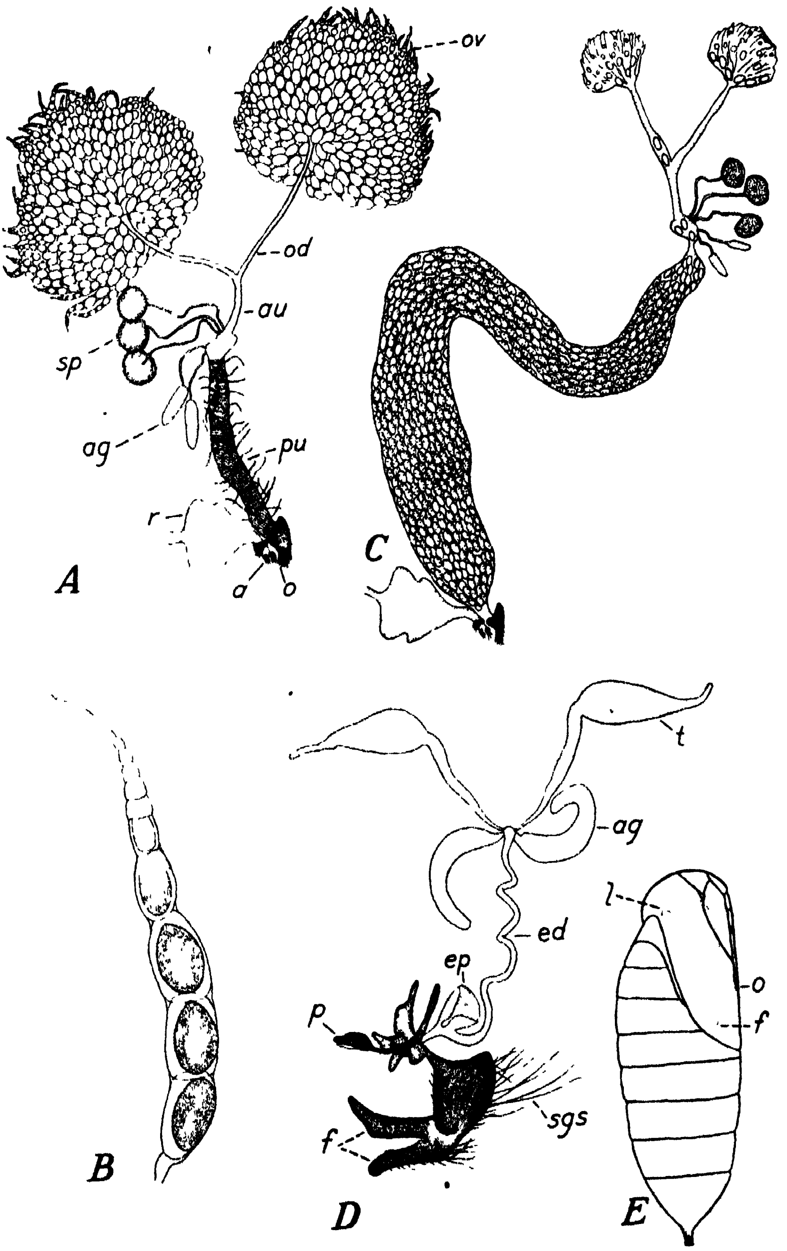

FIGURE 4.—*Zenillia libatrix*: *A*, Reproductive organs of freshly emerged unmated female. *ov*, ovary, *od* oviduct, *au*, anterior uterus, *sp*, spermatheca, *ag*, accessory gland, *pu*, posterior uterus; *r*, rectum; *a* anus, *o*, ovipositor (× 13) *B*, Ovariole (× 38) *C*, Reproductive organs of mated ovipositing female (× 10). *D*, Male reproductive organs *t*, testis; *ag*, accessory gland, *ed*, ejaculatory duct, *ep*, ejaculatory pump; *sgs*, second genital segment, *p*, penis, *f*, forceps (× 17) *E*, Host pupa with *Z. libatrix* larva in situ; *o*, opening to outside air, *f*, tegumental funnel, *l*, parasite larva (× 2).

ovary and 107 in the other. In a freshly emerged female about 10 developing eggs can be seen in each ovariole. If the fly is held several days before mating, all the eggs that will develop can be distinguished. Dissection showed that 14 is the usual number in an ovariole.

After the fly is mated, the eggs develop rapidly. The mature eggs are pushed into the oviducts and pass the spermathecal opening, where they are fertilized, and from there descend to the posterior uterus. The posterior uterus, which is quite short in the unmated female, enlarges enormously as the eggs descend into it until it forms a complete coil, taking up most of the fly's abdomen. Figure 4, *C*, shows the organs of a fertilized female extended in order to show the various parts.

Oviposition does not begin until practically all the eggs are in the posterior uterus. Six females were mated and isolated on July 15. One of them started ovipositing in 8 days, 3 in 9, and 2 in 10 days; but it was not until a few days later that they oviposited eagerly.

The male reproductive organs are shown in figure 4, *D*.

OVIPOSITION

The fact that *Zenillia libatrix* females deposit their eggs on foliage has already been noted. The object of this peculiar habit is to effect entrance into a host larva with the eaten foliage. In confinement the flies oviposit readily on almost any type of leaf, usually along the leaf margin. The fly bends the abdomen forward between its legs and deposits the egg by touching the surface of the leaf. A bit of gelatinous substance on the ventral surface of the egg sticks it to the leaf. The presence of a host larva undoubtedly stimulates oviposition, and if the edges of the leaf are cut the flies will oviposit along these cuts very readily, whether host larvae are present or not. Evidently the flies can sense that a feeding larva is probably responsible for fresh cuts in a leaf.

REPRODUCTIVE CAPACITY

Dissection of several female flies showed that their reproductive capacity varied considerably with the size of the fly. One very small fly had only 820 eggs, while the greatest number found was 2,439. The average number of eggs from 10 flies reared from *Porthetria dispar* was 1,820.

VIABILITY OF EGGS

When *Zenillia libatrix* eggs are laid, the embryos are completely developed, and upon examination distinct movement can be seen. Observations were made to determine how long the eggs that are not eaten remain viable after deposition. Environmental factors affect the eggs so much that it seemed to be impossible to determine the percentage which lived a specific number of days or even their average length of life. Eggs laid on the upper surface of the leaf and exposed to sunlight lost their viability more quickly than those laid on the under surface of the leaf. It was therefore decided simply to try to find the maximum number of days after deposition that eggs would remain so virile that host larvae eating them would become parasitized. For this purpose flies were induced to oviposit on the foliage of trees by confining them for several hours, in small cages, over a few of the leaves. The leaves were then left exposed on the trees and on successive days were cut and fed to host larvae. In 1931, at Budapest, Hungary, when *Porthetria dispar* larvae were fed *Z. libatrix* eggs from 1 to 12 days old, larvae that ate eggs from 1 to 7 days old were killed by the parasite. In 1932, at Melrose Highlands, when larvae of *Bombyx mori* L. were fed eggs from 8 to 22 days old, the larvae that ate eggs from 8 to 15 days old produced *Z. libatrix*. It is therefore apparent that some of the eggs remain viable at least 15 days. Another fact brought out by these observations was that, although the eggs are scraped off the foliage rather easily when a drop of water is applied, they stick on very well during heavy rains.

MODE OF ENTRANCE INTO HOST

The following observations on the entrance of the parasite into its host were made for the writer by D. W. Farquhar:

> By means of the binocular microscope, larvae of *Euchaetias egle* Drury were observed feeding on foliage bearing *Zenillia* eggs. The larvae assumed a position along the edge of the leaf, parallel to its axis, biting off areas of the margin with their laterally operating mandibles. Since the areas bitten off are considerably larger than the eggs, most of the latter are entirely engulfed. However, if the mandibles strike the eggs a glancing blow, the eggs are forced either into the mouth or back onto the leaf to be consumed later If the mandibles strike the eggs a direct blow, the maggot is ejected from the egg and either enters the mouth of the host or adheres to the foliage until subsequently retaken. Although not observed in the laboratory, it is probable that some eggs or maggots, when struck by the jaws of the host caterpillar, drop to the ground and are lost.
>
> An effort was made to locate the portion of the alimentary tract in which the eggs hatched. Dissection of a *Euchaetias egle* larva 5 minutes after it had fed on foliage bearing *Zenillia* eggs showed both maggots and unhatched eggs distributed from the anterior end of the alimentary tract to the large intestine. The maggots were crawling about in the lumen of the canal, none having penetrated the wall. Those eggs that had not hatched yielded maggots readily when lightly compressed with the forceps. An examination of the excrement of parasitized caterpillars showed many empty eggshells but no unhatched eggs. To determine whether the digestive fluids alone were the direct cause of hatching, the entire digestive tract of caterpillars was dissected out, dried externally to remove all blood, slit open, and eggs were introduced. A pronounced swelling of eggs ensued, owing to imbibition of the digestive juices, but none of them hatched.
>
> From these experiments it appears that (1) hatching is not limited to any one part of the alimentary tract but occurs generally throughout its length, and (2) the digestive juices do not alone cause the eggs to hatch, but they induce a swelling of the eggs which renders them more susceptible to rupture due to the variations in pressure caused by motions of the digestive tract and of the caterpillar as a whole. It is probable that the absorption of the digestive fluids is followed by increased activity of the maggot, which assists in the hatching, particularly in the rupture of the vitelline membrane.

The writer also found that *Zenillia* eggs failed to hatch when immersed in fluids removed from the digestive tract of *Porthetria dispar*, but Severin, Severin, and Hartung (*15*) observed that the microtype eggs of *Chaetogaedia monticola* Bigot hatched very well when placed in the alkaline juices emitted from the mouths of several species of host larvae.

LARVAL DEVELOPMENT

The time required for *Zenillia libatrix* to complete its larval development depends more on the development of the host than on the parasite itself. The parasite larva hatches very soon after the egg is eaten and bores through the wall of the alimentary tract into the body cavity. It then enters one of the large abdominal muscles; the silk glands, or occasionally a histoblast. Dissections have shown 46 in silk glands, 37 in muscle fibers, 2 in histoblasts, and 35 "floating free." Those found floating free were probably in muscle fibers that were ruptured by dissection. Larvae have been found in the silk gland 48 hours after the egg was eaten, and it is probable that they reached there some time before that.

The tiny first-instar larva remains in one of these three locations, developing slowly until the host larva starts to pupate. During this period it has no connection with the air and must obtain its supply of oxygen through its body wall or from ingested blood. When the host larva starts to pupate, the parasite larva migrates to the anterior portion of the forming pupa. At this time it starts to grow rapidly, and the basal lobes of the buccopharyngeal armature become sclerotized, as shown in figure 1, *E*. The parasite now forces a small opening in the host pupa between any of the ventral plates of the head sclerites. The irritation set up at this point results in an ingrowth from the host pupa, which rapidly turns dark brown and forms around the parasite larva in the shape of a funnel with the narrow end open to the outside air. This peculiar formation, which is common among the Tachinidae, assures the parasite of a constant supply of air. In *Zenillia* the funnel tube is long and narrow and sharply angled where it widens out (fig. 4, *E*). The wide part of the funnel rests against the inside of the host pupal shell. The rest of the parasite's larval life is completed rapidly. It molts to the second instar soon after the funnel is formed. As it grows the funnel also increases in size. After it molts to the third instar, it rapidly devours most of the contents of the host pupa and issues by cutting an opening between the abdominal segments. Occasionally it forms its puparium inside the host pupa, and the fly issues by breaking the pupal shell.

Because larvae of *Zenillia libatrix* do not begin to develop rapidly until the host larva pupates, the time required for development is variable. Often, when two host larvae are attacked on the same date, one will pupate within a few days while the other may require as long as 2 weeks or more. In such cases the *Zenillia* maggot in the host that pupated may issue and form its puparium while the *Zenillia* maggot in the host larva that did not pupate is still in the first instar. In laboratory rearings 2 *Porthetria dispar* pupae, the larvae of which were fed eggs on August 8, produced *Z. libatrix* puparia 15 and 16 days after attack, and 19 *Stilpnotia salicis* larvae that were fed eggs on August 2 pupated and produced 6 *Z. libatrix* puparia 19 days after attack. In most of the laboratory rearings, though, development was much slower. On August 6, 123 fourth-instar *P. dispar* larvae were attacked, and from them 103 *Zenillia* puparia and 11 *P. dispar* moths were reared. The first puparia were formed on September 1 and the last on September 17. Maximum formation of puparia occurred September 10 and 11, 35 and 36 days after attack.

Zenillia libatrix larvae overwinter in the first or second instar within the funnel formed in their host pupae. Most of the overwintering larvae are in the second instar, but evidently their development is sometimes arrested by cold temperatures before they molt to this instar.

Zenillia libatrix larvae are so tiny (0.23 mm long and 0.10 mm wide) when first hatched that Townsend (*17*) considered it probable that there were 4 larval instars in *Zenillia* and other tachinids laying microtype eggs, as compared with 3 in other tachinids. First-instar *Zenillia* larvae at all stages of development were therefore examined. By comparing the number and arrangement of the cuticular spines, and the size of the anal spiracles and spiracular chambers, it was definitely determined that *Zenillia* has only 3 larval instars. The enormous growth in the first instar is, nevertheless, very striking. Larvae only 0.23 mm long when hatched regularly increase to 10 times this size, or about 2 mm, without molting. Such an increase may, however, be more common than was formerly supposed. Cushman (*6*) found that the ichneumonid *Thersilochus conotracheli* Riley also increases enormously in size during the first instar. He does not give dimensions of freshly emerged larvae, but the egg is only 0.33 mm long and a full-grown first-instar larva is 2.00 mm long.

PERIOD SPENT AS A PUPARIUM

After issuing from the host pupa, the full-grown *Zenillia* maggot burrows into the ground a short distance and forms its puparium. No records of the time between the formation of the puparium and the issuance of the adult flies were made under natural conditions. Table 1 shows the time required when the puparia were held in the laboratory. Records were made in September.

TABLE 1.—*Time spent by Zenillia libatrix in the puparium when held in laboratory*

Time after formation of puparium (days)	Adult males issuing	Adult females issuing	Time after formation of puparium (days)	Adult males	Adult females issuing
	Number	*Number*		*Number*	*Number*
11	4	1	15	0	19
12	7	2	16	0	4
13	18	1	17		1
14	16	15			

As is common among the Tachinidae, the majority of female *Zenillia* issued about 2 days later than the majority of the males. This is an important factor in mating, for the best mating is obtained between freshly emerged females and males from 1 to several days old.

LABORATORY REARING

Zenillia libatrix did not hibernate readily in laboratory-reared material. At Budapest and also at Melrose Highlands, Mass., a large number of host larvae, in which the species develops a summer generation, and which overwinter as pupae, were attacked late in September and early in October. A few of the parasite larvae hibernated, but the majority completed development and the flies issued so late that they could not possibly have found host larvae to parasitize.

Observations were made on the number of *Zenillia libatrix* that could complete development in a single *Porthetria dispar*. Sixty-three host larvae that ate *Zenillia* eggs pupated, and the pupae were isolated. Thirty-seven produced no parasites, 20 produced 1 puparium, 4 produced 2 puparia, 1 produced 3, and 1 produced 5 puparia.

At Melrose Highlands 8 puparia were recovered from a single pupa of *Bombyx mori*.

Fifty *Porthetria dispar* larvae that ate different numbers of eggs of *Zenillia libatrix* were isolated and reared for parasites. In this small number of examples there seemed to be no correlation between the number of *Zenillia libatrix* eggs eaten and the probability that a parasite would develop. One larva that ate 2 eggs pupated, and from these eggs 2 *Zenillia libatrix* completed development; but in a number of instances no parasite developed even when from 10 to 30 eggs were eaten. No superparasitism was detected in these specimens.

It was at first believed that, unless host larvae eating *Zenillia* eggs were fairly large, the eggs would be crushed. It was found, however, that a large percentage of *Zenillia libatrix* completed development in *Porthetria dispar* that were fed eggs when they were small third-instar larvae.

FACTORS LIMITING THE EFFECTIVENESS OF ZENILLIA LIBATRIX AS A PARASITE

The fact that *Zenillia libatrix* has never been reared in large numbers from *Porthetria dispar*, *Nygmia phaeorrhoea*, or *Stilpnotia salicis*, although some puparia are usually recovered from large-scale rearings of each of these hosts, indicates that there must be factors seriously limiting the effectiveness of the species as a parasite. It surely is not the fly's habit of ovipositing on leaves, for *Sturmia scutellata* R.D., one of the most effective of all *Porthetria dispar* parasites, gains entrance into the host in the same manner. Climatic conditions can also hardly be responsible, for *Zenillia* has a wide distribution. *Zenillia* may not be entirely suited to these hosts, but that also hardly seems likely, for when the eggs are eaten under laboratory conditions a fairly large percentage of attacked larvae produce the parasite. No natural enemies of *Zenillia libatrix* were observed, and in *Porthetria dispar* no maggots killed by phagocytes were noted.

A few facts have been noted in rearing work, however, which might be partly responsible for the parasite's low effectiveness. The fact that the species is very polyphagous would seem to limit its effectiveness on any one species. Since *Zenillia libatrix* is double-brooded, a great many individuals complete a generation in the fall after suitable host larvae have gone into hibernation. As these flies perish without reproducing, a small number of flies issue in the spring. Probably the most important limiting factor, though, is that the species must be severely handicapped by parasitic competitors. It requires a long period of development, and host larvae containing small *Zenillia* maggots might be attacked by other parasites, such as *Phorocera agilis* R.D., which would complete development and issue from the host larvae before *Zenillia libatrix* even molted to the second instar. It seems to be just as readily defeated in competition by *Sturmia scutellata*, which usually issues from the host pupa, for this species develops beyond the first instar in the host larva, and therefore when the host pupates it is considerably further developed than *Zenillia*. At Vecs, Hungary, so many puparia of *Zenillia libatrix* were recovered in 1928 that a fair recovery was expected in 1929. As a matter of fact, the species practically disappeared. Apparently it had overwintered successfully, for a male adult was taken in the field that spring. That summer parasitization by *Sturmia scutella* increased enormously.

PROBABILITY OF ESTABLISHMENT

From 1906 to 1910 only 177 adult *Zenillia libatrix* were liberated in New England. In 1927, 327 adults were liberated, in 1928, 1,004 adults, and from 1929 to 1932 only 129 adults were liberated. This is a small number, but, since laboratory work has indicated that the species can hibernate in at least one common native lepidopterous larva, it is quite possible that it has been able to survive. It has never been recovered from collections of *Porthetria dispar*, *Nygmia phaeorrhoea*, and *Stilpnotia salicis* in New England, but perhaps further collections will show that it is actually established.

SUMMARY

Zenillia libatrix is a leaf-ovipositing tachinid, common throughout Europe. It is a parasite of minor importance on the gypsy moth, brown-tail moth, and satin moth and attacks many other species of lepidopterous larvae. It has been liberated in New England but has not yet been recovered.

The various stages of the parasite have been described in detail.

A study of the life history of *Zenillia libatrix* has shown that it has two generations a year and possibly a partial third. The winter is spent as a first-instar or a second-instar larva within the host pupa. The first generation is completed on *Porthetria dispar* or some other host available during May, June, and early July. A second generation may be completed in August, but probably larvae of the second generation hibernate. The parasite has been reared in the spring from larvae of *Pygaera pigra* collected in the field in October.

The adult flies live and mate well in confinement. The females oviposit readily on leaves that have been fed upon or cut. The average number of eggs produced by one female is about 1,800. The eggs may remain viable for as long as 15 days after oviposition. The eggs are eaten by host larvae as they feed upon foliage, and the tiny *Zenillia* larvae hatch and bore their way through the alimentary tract. They enter the silk gland, one of the abdominal muscles, or a histoblast and develop very slowly until the host pupates. They then migrate to the anterior portion of the host pupa, form an integumental breathing funnel, and rapidly finish development. The full-grown larva issues from the host pupa and forms its puparium in the ground. The male usually spends 13 or 14 days in the puparium, the female 14 or 15 days.

As many as 5 *Zenillia* larvae may complete development in a single specimen of *P. dispar*, although usually only 1 parasite issues from a host.

The effectiveness of the parasite seems to be limited by its polyphagous habits, the fact that it is double-brooded, and its slow larval development, which makes it a poor competitor of other larval parasites.

It may be established in the United States, for although only small numbers have been found to overwinter in a common native species, *Melalopha inclusa*, attacked at the laboratory.

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DEVELOPMENT OF A STANDARD CAGE METHOD FOR TESTING THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE[1]

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INTRODUCTION

It is difficult to determine definitely the effectiveness of stomach-poison insecticides on the adult Japanese beetle (*Popillia japonica* Newm.) in the field because of the habits of the insect. The beetle is a strong flier, moving intermittently from one plant to another. A nucleus of beetles on a plant attracts other beetles flying in the vicinity. This insect is generally repelled from foliage on which there is a spray deposit (*4*, *6*, *8*, *13*).[3] When beetles do alight on foliage that has been sprayed with a stomach-poison insecticide, some leave without feeding, some consume a sublethal dose, some consume a fatal dose and fly elsewhere to die, and a relatively small number die on or near the plant. It was early recognized that the beetle would have to be confined and kept under observation before the insecticidal value of a material for it could be determined.

Campbell (*1*), after experimenting with silkworms, proposed a method for determining toxicity based on the introduction of a known quantity of a poison into the body of a feeding mandibulate insect by placing a drop of known concentration in its path and permitting it to imbibe the liquid. This method was tried without success with the Japanese beetle. It did not imbibe readily or completely a drop of liquid placed in its path on a leaf but usually walked through the drop, scattering the liquid over the surface of the leaf. Attempts at forced feeding through the mouth also proved futile because of regurgitation by the insect.

Van Leeuwen (*12*) confined Japanese beetles in individual cages with foliage that was coated with a known quantity of lead arsenate. By measuring the area of leaf eaten he was able to calculate the quantity of poison taken into the alimentary tract. This work indicated that the sandwich method of Campbell and Filmer (*3*) and Campbell (*2*), which overcomes any errors introduced by the material being brushed off the foliage or clinging to the body of the insect and which has been used successfully by Richardson and Haas (*11*) with larvae of the Colorado potato beetle, might be used to determine the smallest quantity of a compound necessary to kill the Japanese beetle.

There are, however, several difficulties connected with testing stomach-poison insecticides against the Japanese beetle in this manner: (1) As each feeding individual must be observed continuously, few materials can be tested at one time and little can be accomplished in the short season that the Japanese beetle is available; (2) the beetle

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[3] Reference is made by number (italic) to Literature Cited, p. 130.

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is a gregarious insect and reacts abnormally when isolated in individual cages; (3) the beetle is an intermittent feeder, moving from one part of the foliage to another and making several punctures which are usually so small that in order to measure them accurately it is necessary to project an enlarged image of the eaten portion of the leaf.

The method by which several insects are confined in each cage with treated foliage and the mortality is determined at intervals seemed to be best adapted for work with a gregarious insect such as the Japanese beetle. As beetles survive in these cages from one to several days, it is not necessary to keep them under constant observation. The investigator is thus able to conduct many tests during the short period in the summer when the Japanese beetle is available for experimentation. This procedure measures the effectiveness or noneffectiveness of a material, but it does not, of course, measure its actual toxicity. The toxicity of a stomach-poison insecticide should be based on the actual weight of the material in the alimentary tract which is fatal to the insect.

TESTING MATERIALS IN WIRE CAGES IN AN OPEN INSECTARY

The procedure that has been used for the last 10 years at the Japanese beetle laboratory has been to confine beetles in a wire cage (fig. 1)[4] in an open insectary with foliage sprayed with a material of known concentration and to compare their death rate with that of beetles confined under the same conditions with untreated foliage. Certain phases of these studies have been published by Moore and Campbell (*9*), Van Leeuwen (*12*), and Fleming (*5*). The results obtained in successive tests with a material by this procedure varied considerably because of differences in the temperature and the relative humidity in the insectary.

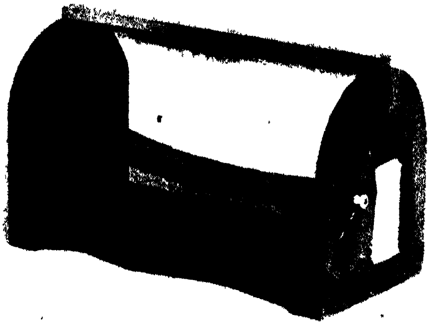

FIGURE 1.—Wire cage used for testing the effect of stomach-poison insecticides on Japanese beetles

PROCEDURE

A series of experiments were conducted to determine the variations in the death rate of Japanese beetles when confined in wire cages and to correlate these variations as far as possible with the environmental factors of temperature and humidity in the insectary. Fifty-one tests were made at daily intervals from July 7 to August 26, 1926, which is practically the period when the beetle can be found readily in the field in southern New Jersey. In each test 100 beetles were placed in two cages with potted plants of smartweed (*Polygonum pennsylvanicum*) that had been sprayed with lead arsenate [5] at a concentration of 5 pounds to 50 gallons of water, 100 beetles were confined in two cages with unsprayed plants of this variety, and the same number were confined in two cages without food. The temperature and relative humidity were recorded hourly between 7 a.m. and 6 p.m., and the beetles alive in each cage were counted at 24-hour intervals for 5 days. At the end of the season the maximum, minimum, and average percentages of mortality of the beetle, when confined with sprayed foliage and when confined without food, were determined by the following formula:

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[4] This cage, which was devised by J. J. Davis, formerly in charge of the Japanese beetle laboratory, is 24 inches long, 12 inches wide, and 14 inches high. The bottom and the ends are of wood. The sides and the top are covered with 16-mesh wire cloth. A hinged door at one end permits the introduction of insects and plants.

$$\frac{\text{Number alive with unsprayed foliage} - \text{number alive with sprayed foliage or without food}}{\text{Number alive with unsprayed foliage}} \times 100 = \text{percent mortality}$$

ACCURACY OF RESULTS WHEN BEETLES ARE COLLECTED IN THE FIELD

In order to have individuals of known age and history, an attempt was made to rear beetles for these tests. A sufficient supply could not be obtained in this manner, so beetles were collected daily, as required, from unsprayed foliage in orchards and fields. It was suspected, because of their feeding habits, that many of these beetles had also fed on sprayed foliage. An experiment was conducted to determine the possible error introduced from this source. At intervals during the summer, beetles were collected from unsprayed foliage on golf courses and farms. Other beetles were reared in the insectary in order to have a group of known history for comparison. One hundred beetles from each group were placed in two cages in the insectary with unsprayed smartweed, and their survival was determined over a period of 5 days. At the end of this period the beetles were digested with arsenic-free nitric and sulphuric acids to destroy the organic matter, and the quantity of arsenic was determined by the standard Gutzeit method.[6] Since arsenic oxide is 32.1 percent of this lead arsenate, the quantity of lead arsenate equivalent to the oxide was found by multiplying by 3.125. Table 1 shows the survival of these beetles and the average quantity of arsenic oxide found per beetle.

TABLE 1.—*The arsenic content and the survival of adult Japanese beetles taken on unsprayed foliage in the field, and of beetles reared in the insectary*

Source of beetles	Beetles in test	Quantity of arsenic per beetle		Survival of beetles				
		Arsenic oxide (As_2O_5)	Equivalent lead arsenate	First day	Second day	Third day	Fourth day	Fifth day
	Number	*Milligram*	*Milligram*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Golf course	500	0.000061	0.000190	99.2	96.6	95.4	94.8	94.2
Cornfield	100	.000150	.000469	100.0	100.0	100.0	100.0	100.0
Grape vine	100	.000156	.000488	100.0	96.0	94.0	94.0	92.0
Hedgerow	300	.000149	.000466	100.0	95.3	93.0	92.3	91.6
Apple orchard	1,400	.000154	.000481	99.3	97.9	96.2	94.0	90.3
Reared in the insectary	300	.000047	.000147	100.0	100.0	96.3	95.0	93.3

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[5] In the tests reported in this paper, ordinary commercial acid lead arsenic containing 32.1 percent As_2O_5 was used.

[6] R. E. Hulse, formerly agent, U.S. Department of Agriculture, made these analyses

All the beetles, regardless of the source, contained some arsenic. The beetles collected on the golf course had probably emerged from the ground only a short time prior to capture, because their arsenic content was practically the same as that of the beetles reared in the insectary. The arsenic content of the beetles obtained on the farms was approximately three times that of the beetles reared in the insectary, indicating that some had fed on sprayed foliage. The average arsenic content of the beetles captured in the field ranged from 0.000061 to 0.000156 mg of arsenic oxide per beetle, which is equivalent to 0.000190 and 0.000488 mg, respectively, of lead arsenate. Van Leeuwen (*12*) has estimated that a dose of lead arsenate between 0.0035 and 0.0156 mg is fatal to the Japanese beetle.

The death rate of the beetles obtained from different sources in the field was practically the same as that of beetles reared in the insectary.

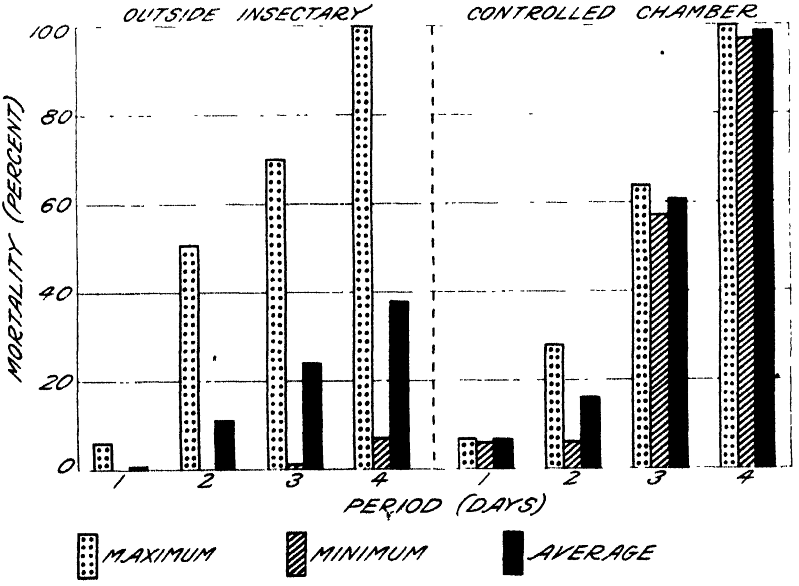

FIGURE 2.—Mortality of Japanese beetles when confined with sprayed foliage in wire cages in an outside insectary and in a chamber under controlled conditions.

It would appear, then, that beetles collected at random in the field from unsprayed plants should be as satisfactory for insecticide experiments as beetles reared in the insectary.

COMPARATIVE MORTALITY OF BEETLES IN CAGES WITH SPRAYED FOLIAGE AND WITHOUT FOOD

The results of the experiment in which insecticides were tested in wire cages in an open insectary are given in table 2 and also, in part, in figure 2. It is seen that at any given period there was considerable difference between the maximum and the minimum mortality. It is, therefore, apparent that the average mortality has no particular significance. Furthermore, since the average death rate of the beetles confined without food in most cases exceeded that of the beetles confined with sprayed foliage, there is some doubt as to whether the beetles in cages with the arsenical died from starvation or from poisoning, particularly since only a small portion of the beetles were on the foliage at any time and the feeding was limited.

TABLE 2.—*Mortality of Japanese beetles when confined in wire cages in the insectary with foliage sprayed with lead arsenate, and when confined in cages without food*

Period of observation (days)	Mortality of beetles					
	Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average [a]	Maximum	Minimum	Average [a]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	6	0.0	0.3	20	0.0	3.1
2	51	0	10.8	50	.0	8.2
3	70	1.0	23.5	100	.0	28.1
4	100	7.2	38.6	100	.0	56.1
5	100	25.0	51.9	100	.0	73.9

[a] As the frequency distributions from which the figures in this column were computed exhibit no definite central tendency, these figures cannot be considered as true averages, but they are included in order to show the discrepancy between them and the valid averages contained in subsequent tables which give the results of experiments carried on under controlled conditions.

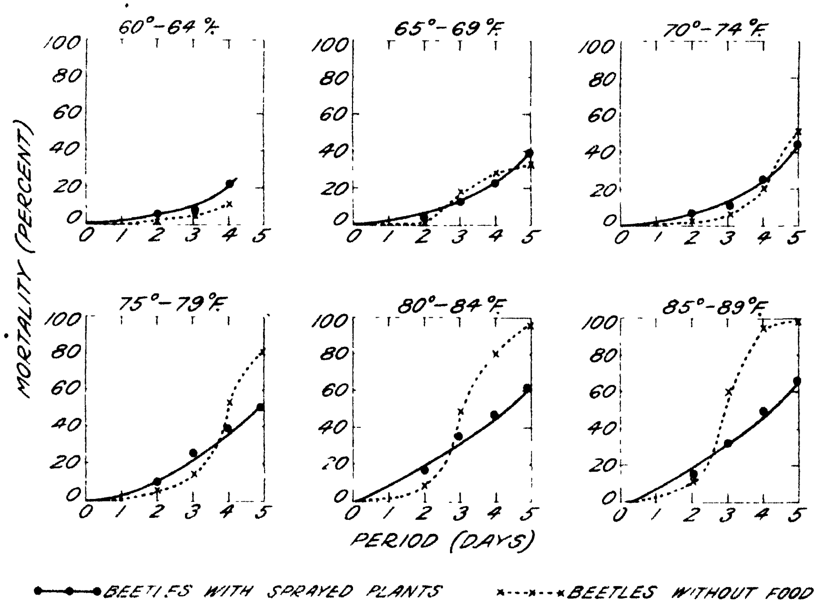

FIGURE 3.—Effect of temperature upon the mortality of Japanese beetles when confined in the open insectary in cages with sprayed plants and in cages without food

EFFECT OF TEMPERATURE ON MORTALITY OF BEETLES

A general idea of the effect of temperature on the death rate in the open insectary was obtained in the following manner: The average temperature for the daylight hours of each day was determined in each of the 5-day test periods in each of the 51 experiments. The beetles surviving in cages with sprayed foliage, with unsprayed foliage, and without food were segregated into groups according to 5-degree ranges of these average temperatures, as follows: 60°–64°, 65°–69°, 70°–74°, 75°–79°, 80°–84°, 85°–89° F. The percentage mortality of the beetles with sprayed foliage and without food was determined for each day in the 5-day period at these temperature ranges according to the formula given on page 117. These data are presented graphically in figure 3. There is a definite correlation between the average temperature of the daylight hours and the death rate. The final average mortality of beetles confined with sprayed foliage was 38.7 percent when the average temperature was 65°–69°, and 65.8 percent when the average temperature was 85°–89°. The average mortalities of the starved beetles under these conditions were 33.4 and 100 percent, respectively. At temperatures below 75° there was close agreement between the death rates of beetles confined with sprayed foliage and those kept without food; at higher temperatures the death rate of the starved beetles exceeded that of the beetles in contact with sprayed foliage after 3.75 days at 75° 79°, 2.75 days at 80°–84°, and 2.5 days at 85°–89°.

EFFECT OF RELATIVE HUMIDITY ON MORTALITY OF BEETLES

The increase in the death rate of the starved beetles above that of beetles with food at temperatures above 75° F. was probably due partly to the low relative humidity at midday which generally accompanies the higher temperatures in this locality. It would be expected that under these conditions, when the insects had no access to water or to a succulent plant, the evaporation on the surface of the bodies might reduce the moisture content to a point where survival was impossible. An attempt to correlate the relative humidity with the death rate was unsuccessful because of the rapid fluctuations in the relative humidity throughout the day. When the beetle had access to a succulent plant, a change in the relative humidity made little difference in the death rate, indicating that there was sufficient moisture in the foliage to maintain the moisture content of the insect's body practically independent of the surrounding atmosphere.

LIMITATIONS OF THE WIRE CAGE IN AN OPEN INSECTARY

The simple wire cage in an insectary, with no control of the atmosphere, is not a suitable environment for obtaining definite information on the effectiveness of different materials as stomach poisons against the Japanese beetle. The principal limitations are as follows: (1) The beetles pass a large part of the experimental period on the wire of the cages in attempting to escape; (2) the beetles do not begin to feed on the foliage until from 20 to 24 hours after introduction into the cages; (3) the fluctuations in temperature, relative humidity, and light modify appreciably the activity and death rate of the beetles, making it difficult to obtain comparable results in successive tests with any material; and (4) the high death rate of the beetles confined without food gives a reasonable basis for questioning whether the mortality in the cages with sprayed foliage should be attributed to poisoning or to starvation, particularly where the feeding of a number of beetles is limited to a few small punctures in the foliage. It seemed imperative to improve this method of testing stomach-poison insecticides before proceeding further with the experimentation.

DEVELOPMENT OF AN IMPROVED CAGE

Early in the spring of 1932 some preliminary experiments were conducted to observe the reactions in different types of cages of Japanese beetles that had emerged in the greenhouses. Some beetles were placed in bell jars with rose foliage. The temperature was 80° F., and the relative humidity was maintained at approximately 70 percent by drawing air through saturated solutions of sodium chloride and then through the jars. A 75-watt bulb was suspended above them for illumination.[7] The beetles did not attempt to escape, as in the wire cages, but fed extensively on the foliage.

A bell jar is not well adapted to insecticide tests on an extensive scale, because of the necessity of aerating it artificially. Several types of cages were constructed in an attempt to retain the desirable features of the bell jars and to eliminate the necessity of drawing air continuously through them. It was found that wire on the sides or top of the cage was not desirable, as the beetles usually went to the wire and ignored the foliage. The beetle is negatively geotropic. It is usually found on the tops of trees, shrubs, and weeds, and when confined in a cage it tends to climb to the uppermost part of any wire or framework to which it can cling, and to remain on any part of the top or sides of the cage, encountered while flying, that can serve as a resting place.

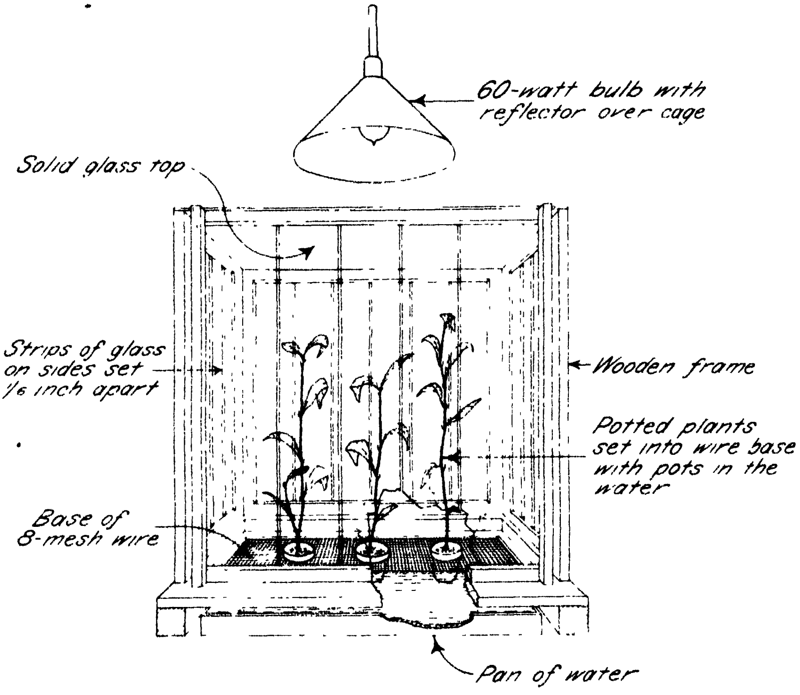
60-watt bulb with reflector over cage
Solid glass top
Strips of glass on sides set 1/16 inch apart
Wooden frame
Potted plants set into wire base with pots in the water
Base of 8-mesh wire
Pan of water

FIGURE 4. Glass cage for testing stomach-poison insecticides against Japanese beetles

From the experience obtained with the different types of cages, a glass cage was constructed which made it practically impossible for the insects to cling to the sides or top and induced them to congregate on the foliage of the plants. This glass cage is illustrated in figure 4. The top is covered with a sheet of glass; the sides are made of 1½-inch strips of glass spaced one sixteenth of an inch apart to provide ventilation. The cage is assembled in such a manner that the wooden frame does not project into the interior, except for a short distance at the top and bottom. The cage rests on a base covered with 8-mesh wire, through which circular holes are cut to receive potted plants. A pan of water is placed under the base. When the plants are placed in the cage, the lower parts of the pots are in the water, making it unnecessary to disturb the beetles to water the plants during an experiment.

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[7] This experiment was carried on by F. W. Metzger, of the Japanese beetle labortory.

DETERMINATION OF OPTIMUM CONDITIONS FOR TESTS

Experiments were conducted in the spring and summer of 1932 to determine the best temperature, relative humidity, and light for carrying on insecticide tests with the Japanese beetle.

OPTIMUM TEMPERATURE

It has been recognized for several years that the Japanese beetle is greatly affected by the temperature of the atmosphere, as it has no precise mechanism for regulating its body temperature. It is generally inactive below 70° F., is most active at 85° to 90°, and is quiescent at temperatures above 100°. From field observation and laboratory experimentation 90° appears to be the optimum for activity. At this temperature a cooling unit is rarely required in a constant-temperature chamber in this locality.

OPTIMUM RELATIVE HUMIDITY

Preliminary experiments were carried on at a temperature of 90° F. and at different relative humidities to determine the optimum humidity to be maintained in the cages. It was found that when the relative humidity was 0 to 5 percent the beetle was generally inactive and fed to a limited extent; at a relative humidity of 35 to 40 percent the beetle fed to a moderate degree but devoted most of its time to flying; and at a relative humidity of 90 to 95 percent it fed extensively and did little flying. These reactions under laboratory conditions are in general agreement with those observed in the field.

The period of survival without food is an important factor in insecticidal work with cages. One thousand beetles were confined without food in a bell jar in which the relative humidity at 90° F. was 0 to 5 percent, another group of an equal number was confined in a bell jar at 35 to 40 percent humidity, and a third group was confined at a humidity of 90 to 95 percent. The mortality of each group of beetles was determined at intervals. The results are presented graphically in figure 5. At a relative humidity of 0 to 5 percent, 50 percent of the beetles were dead at the end of 22 hours; at 35 to 40 percent relative humidity 50 percent were not dead until 48 hours had passed; and at 90 to 95 percent relative humidity 50 percent were not dead until the end of 81 hours. All the beetles were dead in 48 hours at the low humidity, some survived for 96 hours at a humidity of 35 to 40 percent, and some were alive after 144 hours at 90 to 95 percent relative humidity.

The influence of humidity has been ignored in the past in testing the effectiveness of stomach poisons against the Japanese beetle. It may not have so great an effect as temperature, but it does alter the results of successive tests with the same materials. From the results of these experiments it was concluded that a relative humidity between 90 and 95 percent is the optimum for conducting tests with stomach-poison insecticides against the Japanese beetle.

OPTIMUM ILLUMINATION

In the dark the Japanese beetle is practically inactive, although if it is on foliage it may feed a little; it is aroused to activity by illumination. Moore and Coles (*7*) observed that the beetle shows a negative geotropic response only when illuminated. It is well known that the Japanese beetle is affected by the length of the day and the intensity of the illumination. Light is essential to extensive feeding by this insect. Under controlled conditions artificial illumination is necessary. The kind of light to be used in climatic simulation should be determined by the response of the insect. It was planned to study the feeding response of the beetle to artificial yellow light from electric bulbs of different intensities and, if this source of light proved unsatisfactory, to experiment with light from other sources.

The response of the beetle to artificial yellow light of different intensities was studied in the special glass cages in a dark chamber.

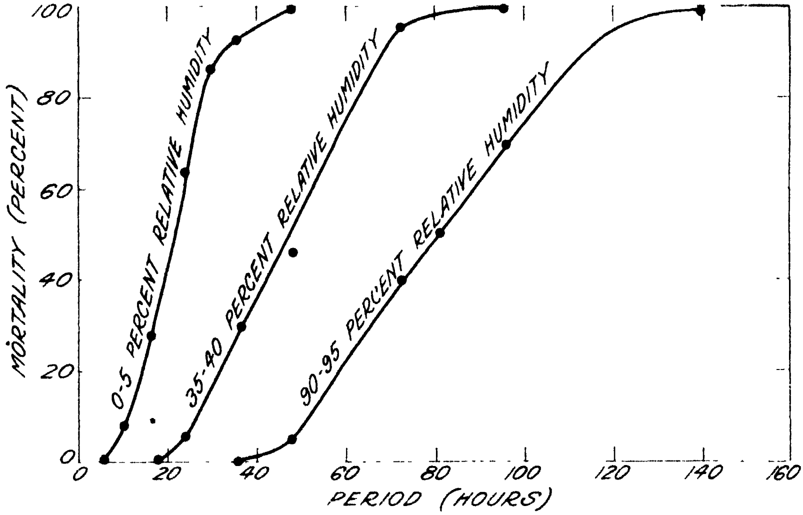

FIGURE 5.—Effect of relative humidity on the mortality of Japanese beetles kept without food at 90° F.

When the average intensity was less than 85 candle-meters, the insect was inclined to be sluggish; with an illumination of 85 candle-meters it moved readily and fed extensively on the smartweed in the cage; but the response was not greatly increased when the intensity was increased to 500 candle-meters. Moore and Cole (*7*) found that the beetle responded in 15.5 seconds to light of an intensity of 85 candle-meters, and by increasing the intensity to 3,276 candle-meters they reduced the time to 10.22 seconds. The activity of the beetles in the special glass cages illuminated with artificial yellow light of an intensity of 85 candle-meters was practically the same as in the field; the beetles collected in groups on the plants, fed extensively, mated freely, and deposited eggs in the soil about the roots of the plants.

This response of the Japanese beetle to artificial illumination is similar to that of grasshoppers, as shown by Parker (*10*), who found that the feeding response of the grasshoppers *Melanoplus mexicanus mexicanus* Sauss. and *Camnula pellucida* Scudd. in controlled cabinets illuminated artificially with a 75-watt bulb was practically the same as the response in the field.

Inasmuch as a satisfactory feeding response was obtained with artificial yellow light of an intensity of 85 candle-meters, it did not appear necessary to experiment with illumination of greater intensity or to try other kinds of light.

An average intensity of 85 candle-meters was obtained by placing a 60-watt bulb in a flood-light reflector 6 inches above the cage. The intensity of the illumination in the cage varied with the distance from the bulb. On the bottom of the cage, 26 inches from the bulb, it was 40 candle-meters; it was 50 candle-meters 4 inches above the bottom, 60 at a height of 8 inches, 80 at a height of 12 inches, and more than 100 at a height of 16 inches. The distribution of light in this manner stimulated the insect to move to the foliage in the upper and lighter part of the cage.

These experiments indicated that the best environment in which to conduct controlled tests of stomach-poison insecticides on the Japanese beetle was one with a temperature of 85° to 90° F., a relative humidity of 90 to 95 percent, and continuous illumination from the top with artificial yellow light having an average intensity in the cage of 85 candle-meters.

CHAMBER WITH CONTROLLED TEMPERATURE, HUMIDITY, AND LIGHT

A satisfactory controlled chamber[8] was constructed by lining the walls and ceiling of a cellar with insulating wall board and installing heating and humidifying devices. The temperature is controlled by a commercial thermostat equipped with a rocking mercury valve. The sensitive element of the thermostat is so arranged that as the temperature falls the mercury flows to one end of a glass tube and closes the circuit between two electrical points; and when the temperature reaches 90° F. the mercury flows away from these electrical points, leaving the circuit open The thermostat activates a centrifugal pump which forces water at 160° through a heating unit and starts a fan which draws air over the heating unit and returns it to the chamber.

The humidity is controlled by means of a silk-thread hygrostat, which by expanding and contracting activates a rocking mercury valve, operating the humidifier. The humidity is maintained at 90 to 95 percent by drawing the air through a fine spray of water and returning it to the chamber.

Each cage is lighted by a 60-watt bulb in a flood-light reflector placed 6 inches above the cage. The walls, ceiling, and benches are painted black to avoid reflection and to have the zone of most intense illumination in the cages.

COMPARISON OF RESULTS WITH LEAD ARSENATE IN THE INSECTARY AND IN THE CONTROLLED CHAMBER

A series of experiments were conducted to compare the results obtained with lead arsenate under these controlled conditions with those obtained in the insectary where there was no control of the atmospheric conditions.

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[8] A. R. Whitcraft, laboratory mechanic, supervised the construction of this chamber and installed the equipment.

In the experiments in the controlled chamber the cages were maintained at a temperature of 90° F. and 90 percent relative humidity and were illuminated constantly throughout the period of observation. Beetles were collected from unsprayed foliage in the field. Fifty were placed in each of three cages with smartweed that had been sprayed with lead arsenate (4 pounds to 50 gallons of water), the same number were placed in three cages with unsprayed smartweed, and the beetles in a third group were confined in three cages without food. Three experiments were conducted during the season, with a total of 1,350 beetles. The number of beetles surviving in each cage was recorded every day for 4 days. At the end of the season the maximum, minimum, and average mortalities of the nine groups of 50 beetles confined with sprayed foliage and also of those confined without food were determined for each 24-hour period of observation according to the previously stated formula. These data are given in table 3 and figure 2.

TABLE 3.—*Mortality of Japanese beetles when confined in glass cages in the controlled chamber with foliage sprayed with lead arsenate and when confined in cages without food*

Period of observation (days)	Mortality of beetles					
	Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	6.5	6.0	6.2	2.0	0	1.0
2	27.6	6.1	16.6	0	0	0
3	64.5	57.0	61.0	28.6	17.8	23.0
4	100.0	97.4	99.0	60.5	38.6	48.8

The results obtained under controlled conditions were compared with those obtained under uncontrolled conditions in the insectary (table 2 and figure 2). It was found that the difference between the maximum and the minimum mortality at a given period was decreased considerably by conducting the experiments under controlled conditions. Whereas the difference between the maximum and the minimum mortality of beetles confined with sprayed plants in the insectary was 6 percent on the first day, 51 percent on the second day, 69 percent on the third day, 92.8 percent on the fourth day, and 75 percent on the fifth day, these differences were reduced to 0.5, 21.5, 6.5, and 2.6 percent, respectively, under controlled conditions. The maximum mortalities for the different periods under insectary conditions, when the temperature was high, compared favorably with those obtained in the controlled chamber. It was therefore believed that, if the experimental conditions in the insectary could have been maintained close to those at which these maximum mortalities were obtained, the results would have been in close agreement with those obtained in the controlled chamber.

When beetles were confined without food, the difference between the maximum and the minimum mortality was greatly reduced under controlled conditions (tables 2 and 3). In the insectary the difference between the maximum and minimum mortality was 20 percent on the first day, 50 percent on the second day, and 100 percent on and after the third day. In the controlled chamber these differences were reduced to 2, 0, 10.8, and 21.9 percent, respectively. The death rate of the beetles confined without food in the controlled chamber, while not so high as that under insectary conditions, is, however, higher than is desired in a test of this type.

In view of the fact that the beetles consumed about 20 percent of the foliage sprayed with lead arsenate under controlled conditions, the mortality due to starvation probably had little influence on the results. It is possible, however, to spray the foliage with a material that will repel the beetles and prevent them from feeding. In such a case the mortality due to starvation becomes an important factor. It was considered desirable, before testing materials as stomach-poison insecticides by this method, to expedite, if possible, the feeding of

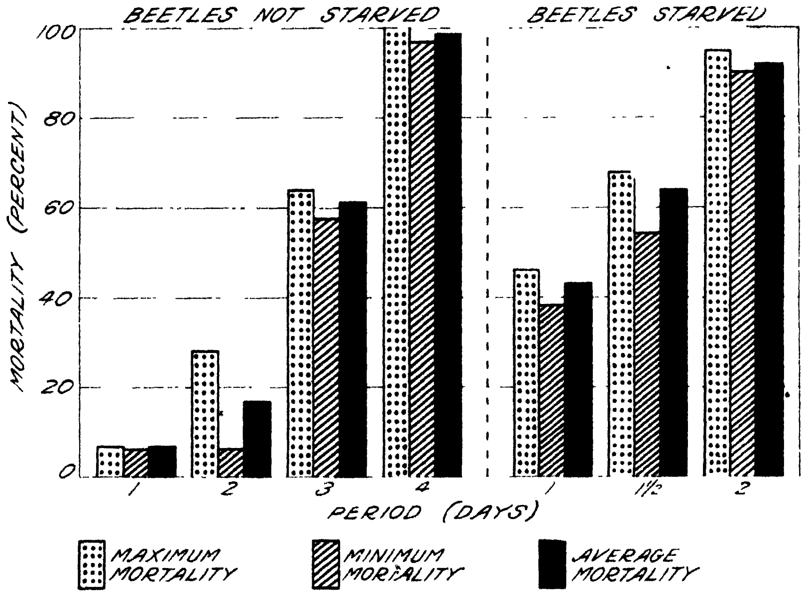

FIGURE 6.—Mortality of Japanese beetles placed in a controlled chamber with sprayed foliage immediately after collecting and after being starved for 3 to 6 hours

the beetles on sprayed foliage and thus increase the death rate to reduce the factor of starvation to a minimum.

EXPEDITING INSECTICIDAL TESTS IN THE CONTROLLED CHAMBER

Beetles collected in the field and placed directly in cages did not gather on the foliage in maximum numbers until 20 to 24 hours after the test was begun. It was obvious that, if beetles could be induced to feed on the foliage in large numbers in a shorter time, the death rate of those confined with sprayed foliage might be expedited. Starving beetles for 3 to 6 hours before placing them in the test cages so stimulated their search for food that 75 to 80 percent gathered on the foliage within 9 hours.

Starved and unstarved beetles were placed in cages with plants sprayed with lead arsenate (4 pounds to 50 gallons of water), in cages with unsprayed plants, and in cages without food to determine the effect of withholding food from the beetles for this period. The number of beetles used was 3,750, from 50 to 400 being used in each test. The percentage mortality of the beetles was determined in the usual manner. The results are given in table 4 and presented graphically in figure 6. With sprayed foliage practically complete mortality of the starved beetles was obtained in 2 days, whereas 4 days was required with beetles directly from the field. Starving beetles for a short time before using them in insecticide tests makes it possible to complete an experiment in half the time required with unstarved beetles. When confined without food for 2 days, the death rate of both the starved beetles and those directly from the field was so low as to be of negligible importance in insecticide tests.

TABLE 4. *Effect of starving Japanese beetles on their mortality when confined in cages with sprayed foliage and when confined without food*

Time (days)	Mortality of beetles directly from field						Mortality of beetles starved 3 to 6 hours					
	Confined with sprayed foliage			Confined without food			Confined with sprayed foliage			Confined without food		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	6.5	6.0	6.2	2.0	0	1.0	46.7	37.7	43.0	---	---	6.4
1½	---	---	---	---	---	---	68.0	53.8	63.3	---	---	4.2
2	27.6	6.1	16.6	0	0	0	94.7	89.8	91.7	---	---	5.0
3	64.5	57.0	61.0	28.6	17.8	23.0	---	---	---	---	---	---
4	100.0	97.4	99.0	60.5	38.6	48.8	---	---	---	---	---	---

NUMBER OF BEETLES REQUIRED FOR A SATISFACTORY TEST

The number of insects used in a test with a stomach-poison insecticide is often taken as an indication of the reliability of the data. It is desirable to use as many individuals as possible in testing a material without making the method cumbersome. In order to determine the consistency of the data obtained with different numbers of beetles, groups of 50, 200, 400, and 500 that had been starved for 3 hours were placed in cages with plants that had been sprayed with lead arsenate. The maximum, minimum, and average mortality of these groups was determined at the end of 48 hours.

The results (table 5) indicate that the number of individuals makes little difference in the average mortality, provided that, when a small number are used, the experiment is repeated several times. The difference between the maximum and the minimum mortality did not change appreciably until 500 beetles were used as a unit. A group of 200 is a convenient unit for tests with the Japanese beetle, and this number is recommended as a standard unit for stomach-poison tests with this insect.

TABLE 5.—*Effect of the number of beetles in a cage on the consistency of mortality tests*

Beetles per cage (number)	Beetles in experiment	Mortality after 48 hours		
		Maximum	Minimum	Average
	Number	*Percent*	*Percent*	*Percent*
50	2,200	94.4	78.4	87.9
200	2,400	95.7	77.3	86.7
400	4,800	94.7	74.1	87.6
500	2,500	89.0	82.9	86.4

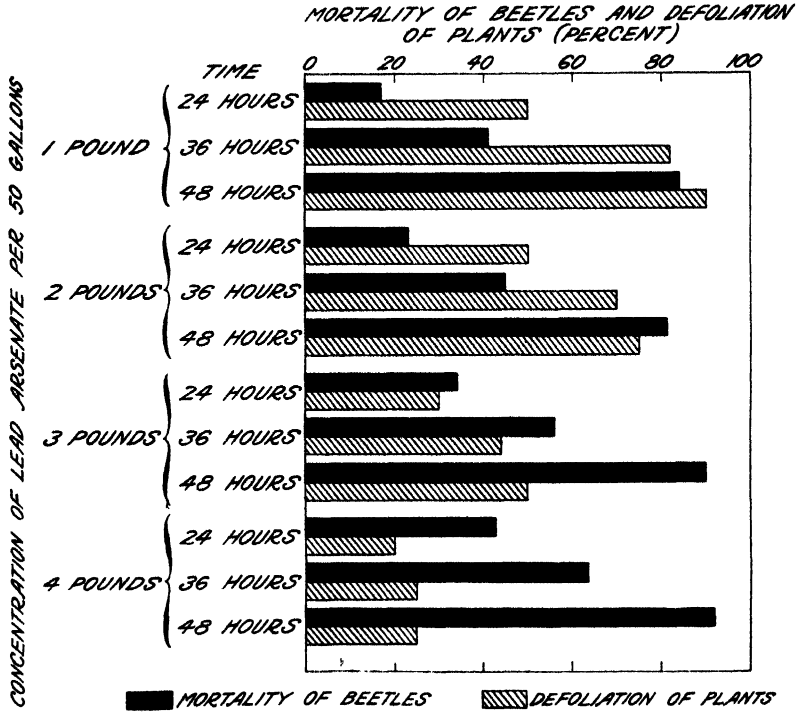

FIGURE 7.—Relation between the concentration of lead arsenate in the spray to the extent of feeding and mortality of Japanese beetles.

RELATION OF CONCENTRATION OF LEAD ARSENATE IN THE SPRAY TO FEEDING AND MORTALITY

A lead arsenate paste was prepared by triturating 50 parts of the powder with 2 parts of oleic acid and adding sufficient water to make the lead arsenate 50 percent by weight of the final product. The addition of the oleic acid gave a more uniform coating without injuring the foliage.

Smartweed plants in cages were sprayed with this paste at concentrations ranging from 1 to 4 pounds of lead arsenate to 50 gallons of water. Two hundred beetles, which were starved as previously described, were placed in each cage with the sprayed plants, and their mortality was determined after 24, 36, and 48 hours. The extent of feeding, as indicated by the degree of defoliation, was also estimated at these intervals. The results of these experiments are given in table 6 and presented graphically in figure 7.

TABLE 6.—*Effect of concentration of lead arsenate sprayed on foliage on the mortality and the extent of feeding of Japanese beetles*

Concentration of lead arsenate (pounds per 500 gallons)	Beetles in experiment [a]	Mortality of beetles									Degree of defoliation		
		After 24 hours			After 36 hours			After 48 hours			After 24 hours	After 36 hours	After 48 hours
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average			
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	3,200	20.6	13.1	17.1	51.9	33.3	40.9	86.4	82.1	84.1	50	82	90
2	3,200	26.7	18.1	22.9	46.8	38.6	44.8	84.4	74.0	81.0	50	70	75
3	2,800	36.6	28.6	33.6	59.1	53.0	56.3	93.0	87.7	90.1	30	44	50
4	2,800	46.5	37.7	43.0	68.0	53.0	63.3	94.7	89.8	91.7	20	25	25

[a] 200 beetles were placed in each cage

The average mortality increased with the increment in the concentration of lead arsenate. At the end of 24 hours there was 25.9 percent difference in mortality between the high and the low concentrations, but after 48 hours the difference was only 7.6 percent. The indications are that if the experiment had been continued for a longer period there would have been no difference.

We may conclude from these data that the Japanese beetle continues to feed on foliage sprayed with lead arsenate until it feels the effect of the arsenic, and that the final mortality is practically independent of the quantity of arsenical residue on the foliage. In order to protect the plants, however, it is desirable that this residue be sufficient to affect the beetle before it consumes much of the foliage.

SUMMARY AND CONCLUSIONS

Testing the effectiveness of stomach-poison insecticides against the Japanese beetle in wire cages, without control of environmental conditions, is not a satisfactory procedure, because of the variation in the temperature, relative humidity, and light, and because the beetle spends a large part of the time on the wire of the cage attempting to escape. A special glass cage has therefore been developed in which there are no resting places at the top or sides and the beetle is attracted to the plants.

Comparable results have been obtained in successive tests with the same materials at a temperature of 85° to 90° F. and a relative humidity of 90 to 95 percent, under artificial yellow light of an intensity of 85 candle-meters.

A group of 200 individuals is a convenient unit for conducting stomach-poison insecticide tests with the Japanese beetle.

The results obtained with commercial acid lead arsenate by this procedure confirm the conclusion that this compound is an effective stomach-poison insecticide against the Japanese beetle. The concentration of the arsenical governs the extent of feeding on the foliage and for a certain period the mortality of the beetles, but at the end of this period mortality is independent of the lead arsenate concentration.

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FROG-EYE (CERCOSPORA DIAZU MIURA) ON STEMS, PODS, AND SEEDS OF SOYBEAN, AND THE RELATION OF THESE INFECTIONS TO RECURRENCE OF THE DISEASE[1]

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INTRODUCTION

The leaf-spotting fungus, *Cercospora diazu* Miura, frequently attacks certain varieties of soybean, *Soja max* (L.) Piper, grown in the Southern States. The name "frog-eye" [2] has been given to the disease produced by this fungus on the soybean. The first authenticated observations of frog-eye on soybeans in the United States were made in 1925, when the disease was found in five Southern States. Announcement of the discovery of the disease in North Carolina and of the probable identity of the causal fungus with *Cercospora diazu* Miura, previously described as the cause of a disease of soybean in Manchuria and Japan, was first made by Wolf and Lehman [3] in 1926. A detailed description of the disease and of *C. diazu* with an account of the isolation and inoculation tests proving the causal relation of the fungus to the disease was published by Lehman [4] in 1928. At that time very few lesions had been found on stems and none at all on pods. Since then many infections of the frog-eye disease have been observed on pods and stems in a number of plantings. In the present paper the writer recounts briefly the symptoms on stems, describes the disease as it appears on infected pods, notes the extent and character of seed infection, gives certain data on longevity of the causal fungus in culture, presents the results of tests showing that the disease may overwinter in leaves and stems left in the field following a diseased crop, and shows that the disease may be initiated in new territory by use of infected seed.

APPEARANCE OF THE DISEASE ON STEMS

Stem infections are less numerous and somewhat less conspicuous than those on leaves. Typical stem lesions have been observed on seedlings growing in large test tubes, but none have been seen on seedlings growing in the field. They appear in the field in large numbers only in late fall when a considerable quantity of inoculum has accumulated on the leaves and the plants are maturing seed. At this time the stems are ripening and are possibly less resistant to fungal invasion than younger stems. Also the lower air temperature and greater abundance of moisture on the plant surfaces at this time of year may constitute conditions more favorable for infection than the hotter summer days earlier in the season.

Stem lesions (fig. 1, *A*) are usually 2 to 4 times as long as broad, the greater dimension lying in the direction of the long axis of the stem.

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[1] Received for publication Aug 3, 1933, issued March 1934.

[2] Lehman, S. G. FROG-EYE LEAF SPOT OF SOYBEAN CAUSED BY CERCOSPORA DIAZU MIURA. Jour. Agr Research 36: 811-833, illus. 1928

[3] Wolf, F A., and Lehman, S. G. DISEASES OF SOYBEANS WHICH OCCUR BOTH IN NORTH CAROLINA AND THE ORIENT. Jour. Agr. Research 33: 391-396. 1926.

[4] Lehman, S. G. (See footnote 2)

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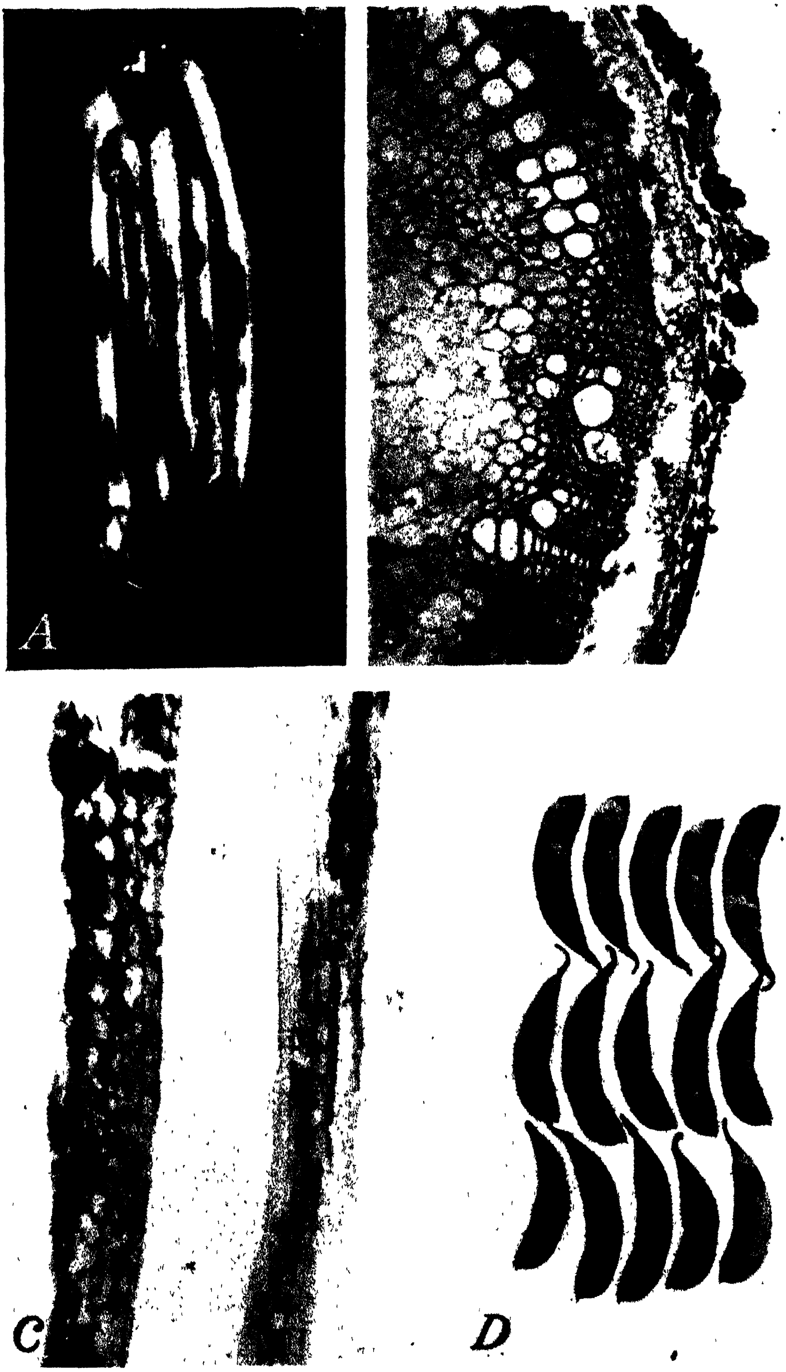

FIGURE 1.—*A*, Stem lesions (yellow filter used in photographing) approximately natural size. The stem of greatest thickness had an actual length of 65 mm. Its color was a ripe brown. The lesion on this stem was 15 mm long and had an outer marginal band of reddish brown, darker than the ripe brown of undiseased tissue. Within this was a narrow band of light brown very little different from undiseased tissue in color. The large central area had been darkened by formation of numerous minute stromata. *B*, Microtome section of stem cut through a frog-eye lesion. Note the darkly stained mycelium in the cortex and three minute stromata protruding through the epidermis. × 100. *C*, An unstained longitudinal section of a stem lesion. Note the pronounced development of mycelium in the cortex and in the phloem beyond the intervening band of sclerenchyma. This section was cut between the lines of a tangent and a radius, thus the sclerenchyma appear excessively thick. *D*, Lesions produced by infection of *Cercospora diazu* on soybean pods. Collected November 1, 1929

They frequently spread one fourth to one half the distance around the infected stem, but seldom does a single lesion girdle the stem. The central portion of the diseased area may be flattened or slightly sunken. On very young lesions the whole diseased area is some shade of red, or the central portion may be red bordered by a narrow zone of dark brown to black beyond which is the normal green of the undiseased tissue. As the lesion enlarges, the central area loses its red color, becoming brown, then pale smoke gray. There may or may not remain a narrow intervening band of red between the gray central area and the dark-brown outer zone. Minute black stromata, sometimes bearing conidiophores with conidia, are often visible in large numbers in the gray centers of old lesions. Sometimes dark-colored mycelium develops so abundantly as to cause the entire surface of older lesions to become black.

Sections cut through diseased areas on stems show an abundance of mycelium in the cortical tissue (fig. 1, *B*, *C*). Mycelium is prevalent in the cortical region including the endodermis. It tends to develop in strands of two to several hyphae, which grow more often in the intercellular spaces than through the cells. Hyphae aggregate in the form of minute stromata at numerous places within or immediately beneath the epidermis. These rupture the overlying cell membrane and extrude as black bodies of pin-point dimensions (fig. 1, *B*). A more or less continuous flattened stromatic development is shown in figure 1, *C*. Just within the endodermis is a layer of sclerenchyma fibers constituting the outer portion of the pericycle. Only rarely are hyphae found in the lumina of sclerenchyma cells or between them. Sometimes the fungus passes through the sclerenchyma and permeates the thin-walled parenchyma of the pericycle and the adjacent phloem and cambium. This passage is made by way of occasional thin-walled cells which separate the several flattened bundles of thick-walled cells constituting the all-but-continuous band of sclerenchyma under the cortex. More often than otherwise the fungus fails to cross the sclerenchyma. Even then, however, the cells of the phloem and cambium lying beneath the invaded cortex are usually found moribund or completely disorganized. Presumably, harmful substances excreted by the fungus growing in the cortex or by-products of the reaction between the fungus and cortical cells diffuse through the sclerenchyma and kill tissues beyond reach of the fungus itself. Likewise, in the cortex the normal condition and the staining reaction of the host cells at the margin of a given lesion are changed for a considerable distance beyond the area actually invaded by hyphae.

LESIONS ON PODS

Lesions have been seen in large numbers on pods of only one variety, the Otootan. This variety does not begin setting pods until late in the growing season, hence pod infections do not become evident until late fall. An earlier fruiting variety, the Laredo, is approximately equal to Otootan in susceptibility of foliage, but comparatively few pod lesions have been found on heavily diseased Laredo plants. This variety matures 30 days earlier than Otootan and at a time when air temperatures are high and relatively little dew accumulates on plants. It is thought that the differences in temperature and humidity, particularly the length of time atmospheric moisture persists on the plants as dew at the time the pods are developing and maturing, may account for this observed difference in the amount of disease found on pods of these two varieties. This belief is supported by the observation that on Otootan the first-formed pods are relatively free of disease as compared with pods formed a week or 10 days later, although many sporulating lesions have long been present on the foliage of the same plants. Usually pod lesions do not become much in evidence until pathological defoliation is well under way. There is also a marked increase in number of cauline lesions at this time.

Lesions on pods are usually quite circular (fig. 1, *D*). However, those which develop in contact with the dorsal or ventral suture tend to enlarge more rapidly along the suture, and those which arise from two or more points of infection and coalesce are irregular in outline. Single lesions vary in diameter from 1 to 4 mm, and as many as 14 have been counted on one side of a single pod. Young lesions on green pods are brown, often with a tint of red. A uniform brown color may persist over the lesion as the spot enlarges, but usually lighter colors develop in the center of the spot, so that on dry, ripe pods the diseased area is seen as a light-brown or gray spot encircled by a narrow dark-brown ring. Thus the so-called "frog-eye" aspect is characteristic of the disease on pods, and to some extent on stems, as well as on leaves.

The surfaces of lesions on pods are usually somewhat sunken because of the collapse of the diseased cells beneath. Figure 2, *A*, a section at the margin of such a lesion, shows the degree of shrinkage in the diseased area. Hyphae advancing within the tissues near the margin of a pod lesion are shown in figure 2, *B*. Nearer the central portion of the lesion, hyphae are much more abundant and tend to form dense stromalike aggregates similar to, but usually smaller than, those found on stems.

When pods bearing lesions of the frog-eye disease are opened, the discoloration marking the location of the infected area may be seen on the inner pod-wall tissues. The pod wall of the soybean is lined with a very thin membrane of hyaline cells. This membrane, which more or less completely invests the seed in each compartment of the pod and lies between it and the pod wall, usually comes free of the seed, leaving the latter smooth and shining when shelled from ripe healthy pods. In diseased pods the portion of this membrance which lies between a lesion and the seed coat often does not shed off from the seed, but clings to the seed coat, marking the point where the seed came in contact with the diseased portion of the pod wall (fig. 2, *D*). This serves as a means of identifying seeds from diseased pods. It is not an infallible sign of seed infection, however, since portions of membranes sometimes adhere to seed coats from other causes than parasitic invasion. Mycelium may usually be found by a microscopic examination of the portion of this membrane lying between the seed coat and the diseased tissue of the pod wall.

Macroscopic examination of seeds from diseased pods reveals no definite lesion or marked discoloration. However, there is often evident a slight depression in the portion of a seed which developed in contact with a pod-wall lesion, and usually the surface of the seed at this place is noticeably lacking in that smoothness and luster characteristic of healthy seed coats. This seed-coat feature, together with adhesion of small portions of the white investing membrane referred to above, appears to be fairly characteristic of seeds that develop in

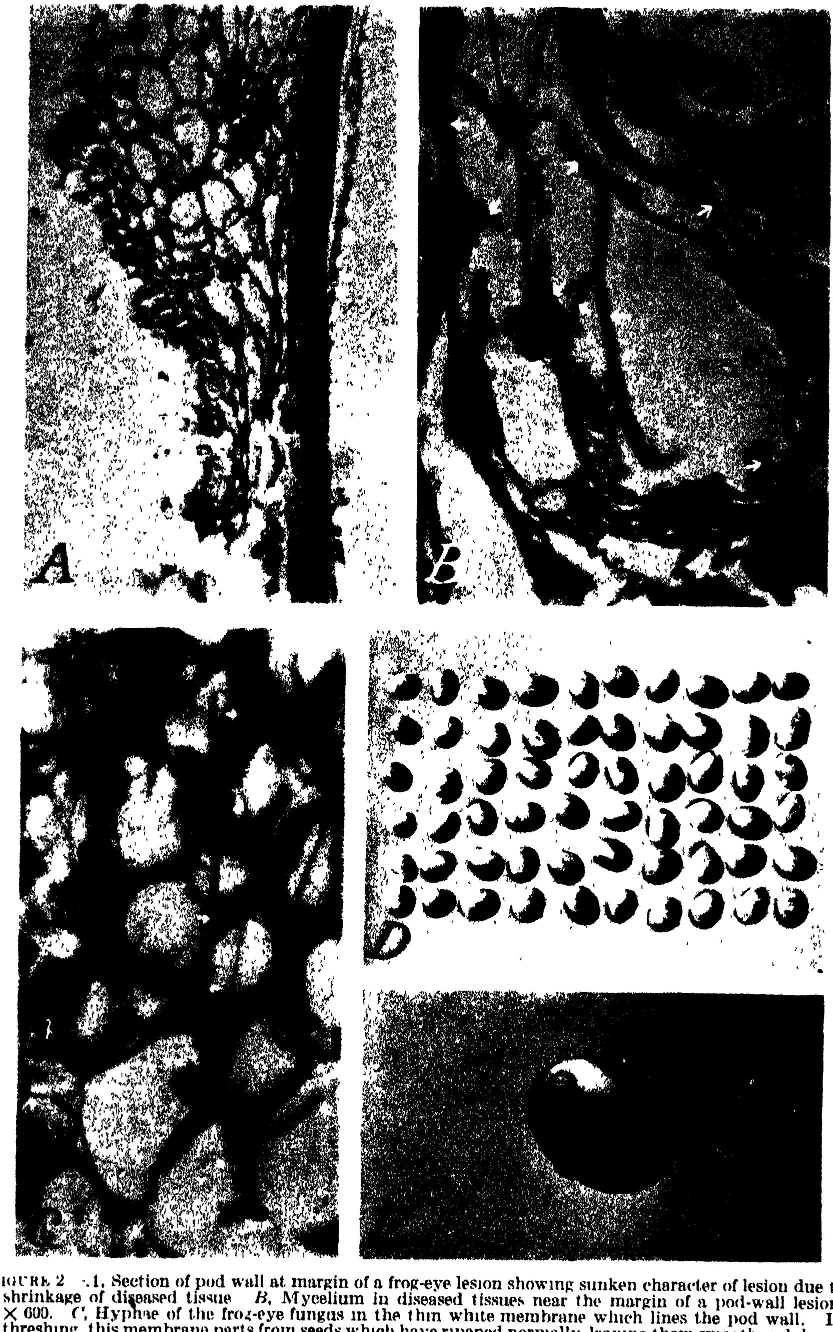

FIGURE 2.—*A*, Section of pod wall at margin of a frog-eye lesion showing sunken character of lesion due to shrinkage of diseased tissue *B*, Mycelium in diseased tissues near the margin of a pod-wall lesion. × 600. *C*, Hyphae of the frog-eye fungus in the thin white membrane which lines the pod wall. In threshing, this membrane parts from seeds which have ripened normally, leaving them smooth and shiny, but portions of it frequently adhere to seed coats at the places where they developed in contact with pod-wall lesions × 500 *D*, A group of soybean seeds taken from pods bearing lesions of the frog-eye disease Portions of the pod-wall lining are adhering pathologically to the coat of each seed This is obscured by high lights on some of the seeds but is plainly evident on others × ⅔. *E*, A soybean showing the depression which often marks the place where the seed lay in contact with a pod-wall lesion None of the diseased pod-wall lining has stuck to this seed. Actual length of seed 6 mm.

contact with pod-wall lesions and may be of some value as a criterion in estimating the amount of infection in different lots of seed. Figure 2, *D* shows a group of seeds that developed in direct contact with diseased areas of the pod wall. The place of contact is indicated either by a depression in the seed coat, or by the adhesion of portions of the thin white membrane, or by both. Figure 2, *E*, shows a single seed enlarged four and one half diameters to give a better view of the seed-coat depression. Macroscopic examination of the portions of the embryo lying in contact with the depressed portions of the seed coat gives no indication that the frog-eye fungus has penetrated embryonic tissues.

ISOLATIONS

Conidiophores and conidia are often seen on pod-wall lesions. In size and shape these are like those of *Cercospora diazu* that develop on leaf and stem lesions. A number of isolations have been made from both stem and pod-wall lesions. The usual procedure has been to wash the diseased portion in tap water, dip momentarily into 95-percent alcohol, immerse 40 to 60 seconds in 1:1,000 mercuric chloride solution, then plant the tissue on plain acidified agar. Nearly all the lesions tested in this way yielded a *Cercospora*, many times in pure culture, but often mixed with other fungi, chiefly *Penicillium*, *Alternaria*, and *Colletotrichum*. The *Cercospora* isolated from stem and pod lesions proved to be morphologically like the strains of *C. dizau* previously isolated from lesions on leaves.[5] The pathogenicity of the strains of *Cercospora* isolated from stems and pods was tested by inoculations on soybean leaves and pods in the greenhouse. These tests are described in a later paragraph.

SEED INFECTION

As previously noted, pod-wall lesions often lie in direct contact with seeds within the diseased pods. No open lesions are visible on the seed itself, there being at most only a slight depression or roughening of the seed coat at the place of contact with the diseased pod wall. Notwithstanding this lack of any conspicuous sign of infection, it seemed probable that seed infection might have occurred. Accordingly, tests were made to determine if the fungus had entered the seeds. In these tests, pods bearing well-developed lesions were selected and only those seeds that lay in contact with pod-wall lesions were used. The pods were washed under the tap, immersed in alcohol for a few moments, removed to mercuric chloride solution, 1:1,000, for 1 to 2 minutes, and finally rinsed in sterile water. The pods were then opened, and the seeds removed with sterile implements and placed on agar media. In certain tests a supplementary disinfection was given the seeds after removal from the pods. Table 1 gives the results of these tests.

The results of tests 1, 2, and 3 show that the frog-eye fungus had passed through the pod wall and had made intimate contact with a high proportion of the seeds selected. Test 3, in which seed coats and embryo were tested separately, indicates that the fungus may have been present on, or in the embryo of, at least one seed. However, since in this test the seed itself was given no disinfective treatment, there remains the possibility that, in the operation of removing the seed coat, mycelium was transferred mechanically from the seed coat to the embryo. The treatments with alcohol and mercuric chloride in tests 4 and 5 reduced the recoveries of the fungus to 1 seed in 11 and 1 in 16, respectively. These tests indicate that the frog-eye fungus lies on or near the surface of infected seeds but may occasionally penetrate to the embryo. Additional evidence of internal infection of seeds will be presented in connection with certain seed-treatment tests described in the following paragraphs.

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[5] WOLF, F. A., and LEHMAN, S. G. (See footnote 3.)

TABLE 1. –*Proportion of seeds yielding Cercospora when taken from diseased pods*

Test no.	Treatment given seeds after removal from disinfected pods	Total seeds	Number of seeds infected	
			On seed coat	In embryo
1	None	7	4	([a])
2	Do.	28	23	([a])
3	Do.	9	9	1
4	95 percent alcohol 5 seconds, $HgCl_2$, 1:1,000, 15–30 seconds, sterile water, 3 changes.	11	1	0
5	50 percent alcohol 20 seconds, $HgCl_2$, 1:1,000, 40 seconds, sterile water, 4 changes	16	1	0

[a] Seed coats not separated from embryos, planted with coats intact

TABLE 2.–*Proportion of seeds yielding Cercospora after harvest from infested field and following certain treatments*

[Germinated in large test tubes on plain 1 percent agar containing 0.2 percent of sugar, seed of Ototan harvested October 1928]

Test no.	Lot no.	Classification	Treatment	Seeds in lot	Germinated	Seeds yielding *Cercospora*	Seeds contaminated with other fungi or bacteria	Seeds originally selected as diseased
				Number	*Percent*	*Percent*	*Percent*	*Percent*
6	1	Strongly diseased	70-percent alcohol 6 minutes, washed through sterile water, 3 changes.	50	98	14	42	
	2	do	None	50	98	2	98	
7	1	Diseased	70-percent alcohol 1 minute, sterile water, 3 changes.	50	90	6	54	13.3
	2	do	$HgCl_2$, 1:1,000, 10 minutes, sterile water, 3 changes	50	98	10	26	
	3	do	70-percent alcohol 1 minute; $HgCl_2$, 1:1,000, 10 minutes; sterile water, 3 changes.	50	98	6	6	
	4	do	40-percent formaldehyde, 1 part in 320 parts water, 60 minutes, sterile water, 3 changes	50	86	0	78	
	5	do	None	50	92	0	100	
8	1	do	$HgCl_2$, 1:1,000, 60 minutes, sterile water, 3 changes.	50	98	4	18	13.1
	2	do	70-percent alcohol 1 minute; $HgCl_2$, 1:1,000, 60 minutes; sterile water, 3 changes.	50	96	4	8	
	3	do	Semesan solution, 0.25 percent, 60 minutes; sterile water, 3 changes.	50	98	6	42	
	4	do	70-percent alcohol 1 minute; Semesan solution 0.25 percent, 60 minutes, sterile water, 3 changes.	50	98	2	8	
	5	Not diseased	$HgCl_2$, 1:1,000, 10 minutes, sterile water, 3 changes.	50	100	0	10	

An attempt was made to determine if one can by inspection recognize the diseased seeds and determine the probable percentage of infestation in a given lot of threshed seed. For this purpose seed known to have come from an infested field were used. The usual procedure was to take at random one or more handfuls of seed from the bag. This seed was then sorted into two lots, one containing all seed judged to be diseased, the other those judged to be disease-free. The criterion of infection or contamination was the presence of a dulled, slightly roughened, often slightly depressed area on the seed coat. To this area a fragment of the thin white pod lining might or might not be clinging more or less tenaciously. The seed were germinated in large cotton-plugged test tubes containing plain agar media with 0.2 percent of sugar. The results of these tests are given in table 2.

In test 6, 100 seeds which showed the most pronounced evidences of disease out of a total of 576 were selected. The frog-eye fungus grew from 14 percent of the seeds which had been given the alcohol treatment. The untreated seeds of lot 2 were to all appearances equally as badly diseased as those of lot 1, but the frog-eye fungus appeared on only 2 percent of them when put to germinate although they had been given no preliminary disinfection. This unexpected result was probably due to the suppression of the comparatively slow growing frog-eye fungus by the heavy cultures of contaminating fungi in the tubes of lot 2. A much higher percentage of the tubes was contaminated in lot 2 than in lot 1 as was to be expected since lot 2 had received no treatment with a disinfectant.

The random sample taken for test 7 contained 2,138 seeds. When this sample was sorted, 286 seeds, or 13.3 percent, were found to bear the seed-coat markings taken as evidence of their having come from diseased pods. These diseased seeds were then divided into 5 lots of 50 seeds each and given the treatments indicated in table 2. In this test, lots 1, 2, and 3 yielded 6, 10, and 6 percent of diseased seed, respectively. The frog-eye fungus was not recovered from the seeds, of lot 4 nor from lot 5. Here again failure of the frog-eye fungus to appear in the untreated lot 5 was probably due to its complete suppression in competition with other contaminating fungi and bacteria. In lot 4 both the inhibiting action of contaminating organisms and the toxic action of formaldehyde may have operated to prevent the appearance of the frog-eye fungus. Judged by the high percentage of tubes infested by contaminating organisms, the formaldehyde treatment was not an effective seed treatment, yet its action of formaldehyde may account for failure of the frog-eye fungus in the uncontaminated tubes.

In test 8 a random sample containing 2,080 seeds was taken. Of these, 13.1 percent, or 273 seeds, showed the seed-coat symptoms, indicating that they had come from diseased pods. Four lots of 50 seeds each were taken from the diseased seeds. As indicated in table 2, the diseased seed were given somewhat more severe treatments than in previous tests in order to determine if the fungus might be inactivated in all the diseased seeds. Lot 1 was soaked in 1:1,000 $HgCl_2$ solution for 60 minutes, lot 3 in 0.25 percent Semesan solution for 60 minutes, and lots 2 and 4 were given a 1-minute soaking in 70-percent alcohol preliminary to the 60-minute soaking in the mercuric chloride or Semesan solution. The frog-eye fungus grew from 4, 4, 6, and 2 percent of the seeds in lots 1, 2, 3, and 4, respectively. Lot 5 consisted of 50 seeds from the undiseased portion of this seed sample. This lot was also treated with mercuric chloride solution to check development of contaminating fungi in the germination tubes. Frog-eye fungus was not obtained from any seed in this lot.

The results of the seed-treatment tests show that it is possible to determine by inspection whether or not any considerable proportion of the pods from which the seeds came were infected with frog-eye. However, the percentage of threshed seeds from which the frog-eye fungus has been recovered in these tests has been low. For example, in test 7 (table 2), 13.3 percent of a sample taken at random were judged by inspection to be diseased. In the germination test only 6 percent of the seeds in lots 1 and 3 and 10 percent in lot 2 produced cultures of the frog-eye fungus. This is equivalent to only 3.85 percent of the 286 seeds selected as obviously diseased. Evidently this low percentage of recovery of the frog-eye fungus is due to the use of the seed disinfectant before putting the seed to germinate and does not mean that the criteria used in identifying diseased seeds are untrustworthy. This explanation is supported by the results given in table 1. In tests 1, 2, and 3, the seeds themselves were given no disinfecting treatment but were taken from the interior of diseased pods which had been treated with disinfectants before opening. *Cercospora diazu* grew from an average of 81.8 percent of these seeds. On the other hand, this fungus grew from less than 10 percent of the seeds in tests 4 and 5, in which the seeds were taken from beneath pod-wall lesions but were treated before being put to germinate. The use of the disinfectant not only more or less effectively controls contaminating fungi but also kills the frog-eye fungus on a considerable proportion of the seeds.

It is desirable to make germination tests on agar media. The seed sprout well on moist cotton or other nonnutrient media, but here the fungus on many seeds does not find enough nutriment in the seed-coat tissues to permit development to a point where it can be identified. On the other hand, this fungus often grows where the infected seed has come in contact, even though only momentarily, with moist agar when it does not develop on the seed coat. When seeds can be taken directly from pods the agar tube method gives satisfactory results; but when threshed seeds are used, contaminating organisms so overgrow and suppress the slow-growing frog-eye fungus as to make satisfactory results with this method impossible. The use of nutrient agar as the medium of germination makes it necessary that the seeds be given a preliminary treatment with a surface disinfectant to suppress contaminating organisms, and this in turn undoubtedly destroys the frog-eye fungus on that portion of the seeds that are only superficially infected. The infection percentages obtained by this method of seed testing must be taken to represent only a small fraction of the seeds with which the frog-eye fungus is in intimate contact. The seed tests indicate that in a relatively large percentage of the seeds the infection is so superficial as to be killed by disinfectants. This infection is real, however, and may initiate disease when untreated seed are planted. Experience indicates that where as many as 10 percent of the seeds show visible marks of disease practically 100 percent of the leaves of the plants from which the seed came were infected, each with numerous lesions.

The tests recorded in table 2 constitute additional evidence of the nature of seed infection. Such severe treatments as 70-percent alcohol for 1 minute followed by soaking in 1:1,000 solution of mercuric chloride for 10 and 60 minutes, and by 0.25 percent Semesan for 60 minutes failed to eradicate the disease. The fungus in a small percentage of the seeds is evidently located deep within the seed-coat tissues, between the seed coat and embryo, or actually in the tissues of the embryo. Tests 3, 4, and 5 indicate that penetration of embryo tissues occurs in only a very small percentage of cases, if at all.

INOCULATIONS

Upon the discovery of frog-eye disease on stems, pods, and seeds, and the subsequent isolation of *Cercospora diazu* therefrom, it seemed desirable to use these isolants in the production of the frog-eye disease on leaves, and, conversely, to use isolants from leaf lesions in the production of the disease on pods and seeds. Accordingly a number of inoculation trials were made. The first of these were made with cultures isolated from seeds and pod-wall lesions on the unifoliate leaves of Otootan seedlings. The inoculations were made by rubbing the fruiting surface of the fungus colonies growing in Petri dishes over the surface of the leaf. This was done gently so as to sow the leaf surface with conidia but leave little or no mycelium thereon. One unifoliate leaf of the pair on each plant was inoculated, the other of the pair being left as a check. The inoculated plants were kept in a humid atmosphere under bell jars for 3 days, after which time the bell jars were removed. All leaves, 4 in number, thus inoculated with isolants from pod-wall lesions, and all leaves, 8 in number, inoculated with isolants from seeds produced numerous lesions typical of those caused by the frog-eye disease on leaves in the field. By the end of 20 days from the date of inoculation some of the inoculated leaves were becoming chlorotic and were dying from the large number of lesions. The uninoculated check leaves, paired on each plant with inoculated leaves, were healthy, green, and larger than the corresponding inoculated leaves.

In another test, inoculation with cultures isolated from pod-wall lesions were made on several leaves of a large plant of the Biloxi while uninoculated leaves remained healthy. Successful reisolations variety. Typical frog-eye spots developed on the inoculated leaves, by approved methods were obtained from 7 out of 9 lesions taken from the inoculated leaves.

In a third test, a culture isolated from leaves and another isolated from stems were used in inoculating pods, stems, and leaves. A spore suspension was made by washing the surface of a tube culture with 5 cc of tap water. This was then applied to the parts inoculated by gentle rubbing with the fingers. Check plants were rubbed in the same manner, only tap water being used for this purpose. At the end of 19 days numerous typical lesions were present on leaves inoculated by spore suspensions from each of the cultures, while check plants growing in the same pots remained free of disease. No pod or stem lesions had developed by the twenty-third day, but slightly more than half the inoculated pods had died. Since this had also occurred on the check plants the dying of these parts was taken to be due not to disease but to injury in the operation of inoculating or to ineffectual pollination. When the plants were examined again 8 days later, 6 out of 17 of the remaining inoculated pods bore disease spots faintly or closely resembling those on infected pods in the field, while pods on check plants remained free of disease.

The diseased pods produced by the artificial inoculations described were used in reisolation tests. The pods were dipped for 10 to 15 seconds in 95-percent alcohol, then for 1 minute in 1:1,000 mercuric chloride, and finally rinsed in sterile water. The lesions were cut out and placed on potato agar. Also a number of the seeds which lay directly beneath pod lesions were removed with sterile implements and without further sterilization placed on nutrient agar in test tubes. *Cercospora diazu* was obtained from 9 of the 14 pod-wall lesions used. The plates in which the remaining 5 pod-wall lesions were planted became overgrown with *Rhizopus* sp. before the frog-eye fungus had time to develop in them. Three of eight seeds taken from these artificially inoculated pods yielded cultures of the frog-eye fungus. The mycelium of the fungus was quite insecurely attached to the seed coats, for colonies of the frog-eye fungus arose not only on the seed coats but also at several places where the infected seed touched the surface of the nutrient medium in rolling down the slope of the agar slant in the tubes used.

LONGEVITY OF THE FROG-EYE FUNGUS

In a previous paper[6] the author pointed out that conidia of the frog-eye fungus can survive long periods of storage in an air-dry condition. It was shown that a small proportion of the conidia were still viable when taken from leaves which had been kept dry in the laboratory for 94 days from the date of collection. Observations not previously reported show that the mycelium of the frog-eye fungus is also very long-lived in culture media. In making transfers of the fungus, old cultures have at various times been used. The usual procedure in making new cultures was to transfer a small quantity of mycelium and such spores as might be present either from the surface or edge of the old colony to fresh media. On one occasion transfers were made in this way from cultures which had been for 395 days on potato-glucose agar in tubes of 18 mm internal diameter. After the lapse of several days these transfers showed no evidence of growth. The entire quantity of substratum and mycelium of the old cultures was then removed and pressed into fresh potato-glucose agar in tubes. When transfers of the old cultures, now 405 days old, were made in this way, 9 of 10 cultures were found to be viable. At the time these transfers were made the old cultures were to all appearances thoroughly air-dry and occupied only a small fraction of their original volume. The cultures usually attain this air-dry condition in the course of 120 to 180 days. Table 3 shows the results of this and other tests of a similar nature. The frog-eye fungus has been found still viable in cultures as old as 493, 502, and 519 days. Three cultures which had reached an age of 919 days, and 15 which ranged from 979 to 1,731 days could not be revived by the method used here. The hot-agar method described by Povah[7] was used without success on five cultures ranging in age from 1,001 to 1,768 days. Apparently under the conditions of this test the frog-eye fungus will live on potato-glucose agar, and perhaps also on cooked rice, as long as, or longer than 519 days, but not so long as 919. It is judged that during approximately 400 of the 519 days the fungus remained alive, the substratum in which the fungus was embedded contained only such meager quantities of water as could be held against evaporation in the dry air of the laboratory. It seems probable that the fungus might live equally long in diseased leaf and stem tissues constituting the debris left from a diseased crop.

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[6] LEHMAN, S. G. See footnote 2.

[7] POVAH, A. NOTES UPON REVIVING OLD CULTURES. Mycologia 19: 317-319 1927.

TABLE 3.—*Longevity of Cercospora diazu in culture*

Strain or isolation no	Age at time of transfer	Cultures transferred	Cultures still viable	Strain or isolation no	Age at time of transfer	Cultures transferred	Cultures still viable
	Days	*Number*	*Number*		*Days*	*Number*	*Number*
1 Re 1c	221	2	2	2-4	519	1	1
4C	341	1	1	10	919	1	0
1-3	405	4	4	Re 1	919	2	0
1-3 [a]	405	4	4	1	979 (and older)	15	0
2-2	405	2	1	7 [b]	1,001	2	0
1-4-5	493	2	2	4C 4 [b]	1,768	3	0
1-4	502	4	4				

[a] Growing on cooked rice, all other cultures grew on potato agar containing 2 percent of glucose or dextrose

[b] Hot-agar method

OVERWINTERING OR SEASONAL SURVIVAL ON INFECTED LEAVES AND STEMS

Observations made early in the writer's experience with frog-eye of soybean gave rise to the belief that the causal fungus could survive from one growing season till the next on debris left in the field from a previous diseased crop. To test this hypothesis, diseased leaves were collected in the fall and stored out of doors in wire baskets. These were examined at various times during the winter to determine if the frog-eye fungus was still alive. On February 27, many conidia and conidiophores were present on the frog-eye spots on these leaves. The conidiophores were standing in large fascicles and appeared to be growing. When put to germinate in tap water at room temperature, many of the conidia produced 1, 2, and 3 long germ tubes overnight. The stored material was examined again on May 2. Decay had progressed so far that the leaves could not be handled without breaking apart. However, conidia of *Cercospora diazu* could still be found in small numbers, and these appeared to be in a viable condition capable of germination and infection if transferred by wind or other agency to living leaves.

The overwintering of the soybean frog-eye disease on diseased leaves was further tested in the following way: In October 1928 approximately a bushel of diseased leaves were gathered up from the ground beneath soybean plants. These leaves were stored in an orange crate out of doors under some tall shrubbery. On August 20 of the following year, seed of the Otootan variety of soybean were sowed in a garden in which soybeans had not grown in many years and which was nearly 1 mile from the nearest soybean field. The seed used in sowing this garden were harvested the previous season from a variety plot that had been inspected and observed to be free of frog-eye. When the seed was sowed on August 20, the overwintered diseased leaves were scattered about over a part of the bed. Although occasional light rains occurred, the weather remained comparatively dry and was not judged to be particularly favorable for development of fungi in the diseased leaves lying on the ground where they were subject to rather rapid drying in the hot summer days. Conditions for sporulation of the parasite on the old leaves became more favorable, however, after the seedlings developed sufficient foliage to shade the ground. The first lesions were found on September 26. These were on the lower foliage on the part of the bed over which the diseased leaves had been scattered. Later the disease spread to all parts of the bed.

There still remains the possibility that in this test the plants became infected from wind-blown spores from a diseased field approximately 1 mile away. This seems improbable, however, in view of the following facts: (1) The disease appeared first on the part of the bed to which the overwintered leaves had been applied; (2) the disease did not appear on the varieties Herman and Virginia which had been growing for a much longer period in an adjoining garden; and (3) the disease accumulated comparatively less rapidly in Otootans growing for the entire season less than one tenth mile from the field in which spores were being produced, and between it and the garden in which this test was made. It is highly probable that the diseased leaves saved from the previous season served as the source of inoculum for the beans in the garden test.

On March 26 an examination was made of old stems and pods left in the field after the harvest of a diseased crop of Otootan soybeans in the previous fall. Conidia were readily found on old lesions on both pods and stems. The conidiophores were present in clusters of several to many short individuals. Most of them had at that time formed only one conidium, but many of them were growing forward as they characteristically do before the formation of a second conidium. In size, shape, and septation the conidia found on these overwintered lesions were like those previously observed on diseased leaves. At this time numerous soybean seedlings were coming up in this field from seed which had fallen to the ground when the beans were harvested in the fall. Some of these had unfolded their first leaves—the unifoliate pair. No lesions could be found on these leaves at this time—March 26—but when the field was examined 2 weeks later young frog-eye lesions were present on leaves of approximately 0.5 percent of the seedlings. Later observations could not be made because the field was plowed up at this time. Doubtless some of these germinating seed had come from infected pods, and it may be that the infections present on the leaves of some of the seedlings arose from primary seed infection. However, no seedlings were found, after careful examination, on which there was any evidence, such as growth of the fungus on cotyledons, stem, or seed coat, that the leaf infection had arisen from seed infection. On the other hand, it seemed far more likely that the infection on these seedlings had arisen from conidia produced on overwintered debris from the previous diseased crop.

It often happens, that in plowing the soybean stubble, the diseased stems are only partly turned under, many of them sticking out of the soil between furrows. This is likely to occur when the old stems are so long as to accumulate under the plow beam and slip out in large bundles. In such fields one may often find the frog-eye fungus sporulating in late March or early April. Numbers of volunteer seedlings are also often present that have frog-eye lesions on their first-formed leaves.

A small field in which a badly diseased crop of soybeans had grown in 1928 was divided into two portions that were plowed up at different times. Numerous lesions had been present on leaves, stems, and pods. The beans were harvested in late October, the stems, pods, and leaves being left on the land. Plot 1 was broken with a turning plow on December 1 and plot 2 on April 25. In this operation the diseased stems, pods, and leaves were turned under, not completely, but very much more so than is accomplished in the usual plowing operation. On May 15 both plots were thoroughly disked and planted, Otootan seed from a disease-free field being used. The disking destroyed the volunteer seedlings which had come up previous to planting on May 15. The beans were planted in rows, and notes on the presence of frog-eye were taken from time to time as the season advanced.

The first notes were taken on June 13, at which time the plants, having shed their cotyledons, possessed two fully expanded trifoliate leaves in addition to the usual pair of unifoliate leaves and a third folded trifoliate leaf. On plot 1, at this time, it was estimated that 10 percent of the plants had frog-eye lesions on their unifoliate leaves, there being usually 1, but sometimes 2, 3, and 4 spots per individual leaf. A single lesion was found on a trifoliate leaf. A very few volunteer plants had come up tardily between the rows, apparently from the slow germination of seed having hard coats, but none of these were diseased. Several scattered plants which obviously had germinated before the land was disked for planting and had not been killed by this operation, were present in the plot. These could be identified readily by their larger size. Only one of these showed frog-eye lesions. This one plant could not have been responsible for any considerable proportion of the lesions on the other plants. It seems a safe conclusion that the disease on most of the plants which came from disease-free seed planted on May 15 resulted from spores produced on the overwintered debris.

Plot 2, which had been turned under in April, was separated from plot 1 by 20 rows of corn. Careful observation indicated that there were not appreciably more plants showing infection of unifoliate leaves on plot 2 than on plot 1. Lesions were found, however, on several trifoliate leaves of plot 2, as compared with only one on plot 1, but the difference in number seemed so meager as to be without significance. Later observations revealed no difference in amount of disease on the two plots. By August 15 all plants were infected, 75 percent of the leaves showing one or more lesions. The test demonstrates satisfactorily that the debris from diseased fields may harbor the disease till the following spring and that this debris is not plowed under sufficiently well in the usual farm operations to prevent recurrence of the frog-eye disease even though disease-free seed is used.

FROG-EYE FUNGUS ON DISEASED SEED

The presence of the frog-eye fungus on and within the seed coat of beans taken from diseased pods has been amply demonstrated in the foregoing paragraphs. Evidence that the use of diseased seed is responsible for the appearance of frog-eye in new fields or new communities is found in the following tests: Although observations were being made over a period of several years in connection with the study of other soybean diseases, the frog-eye disease was not seen in the field in the vicinity of Raleigh until 1927. In that year, seed of the

Biloxi variety, produced in 1926 in a distant part of the State in a field known to be infested with frog-eye to a moderate degree, was planted at Raleigh. Seed of the Laredo variety likewise obtained from a distant part of the State was also planted at Raleigh. A portion of each kind of seed was treated with chemical disinfectants, the remaining portion being left untreated as a check on the treatments. Plantings were made May 25. No frog-eye lesions could be found on July 6 or August 3. On August 17 a small number of lesions were found scattered at irregular distances along the check rows of both Biloxi and Laredo, while none was found at this time on rows which had been planted to treated seed, although there were 1,900 feet of treated rows, as compared with 1,000 feet of untreated rows. It seems obvious that the disease originated in this field from infected seed. If it had been introduced by means of spores carried long distances by the wind, lesions should have been equally prevalent on all rows in this test. Observations indicate that, at the early date at which the first infections were noted in this test, the fungus had not produced a sufficient quantity of spore material to account for any but the most meager aerial spore population.

In another test, Otootan seeds harvested from diseased plants and here designated as lot 1 were planted at one side of a field which had been in cotton the previous year. Lot 2, which was planted on the opposite side of the field, consisted of Otootan beans from a different source, but selected with the expectation that they were disease free. This planting was visited for taking notes on August 6, approximately 3 months after the beans had been planted. At that time frog-eye was found at scattered locations along the rows planted with the infected seed (lot 1), but none was found on the rows planted with disease-free seed (lot 2). On the plants of lot 1, the lesions were rather numerous at a few locations along the rows as one would expect if the first secondary infections had resulted from the germination of diseased seed at these particular places. At these places there were many leaves with several spots per leaf, and a majority of the lesions were on leaves at the middle or bottom of the plants. Between these centers of dense infestation there was a sparse distribution of lesions on upper leaves. These lesions were initiated presumably from spores produced at the places of primary seedling infection. Since in this test frog-eye occurred only on plants from infected seed of lot 1 and not on plants from the seed of lot 2 which were equally exposed to possible wind-borne infection, it is believed that the disease originated from fungi carried to the field in the seed of lot 1.

The exact course taken by the frog-eye fungus in passing from the infected seed to the foliage of the plant which grew therefrom has not been satisfactorily determined. It might be expected that the fungus would grow in and sporulate on the tissues of the expanded cotyledons of the seedling, the spores passing from the cotyledon via the air to the unifoliate leaves or the stem and finally to trifoliate leaves formed later. However, the writer has never been able to find cotyledonary lesions unquestionbly those of the frog-eye fungus on plants in the field. Isolations have been made from suspected lesions; but none has yielded the frog-eye fungus, while little or no difficulty has ever been experienced in isolating this fungus from leaf, pod, or stem infections. When infected seed is germinated on agar in test tubes, the fungus often becomes visible only at places where the seed has touched the agar. Usually in test tubes the seed coat does not shed off the seed leaves but often clings to one or both of them as they are elevated into the air by the elongating hypocotyl. The epicotyl may elongate, pushing the plumule out from between the cotyledons which are held rather firmly together by the adhering seed coat. The frog-eye fungus often grows out and sporulates on the surface of infected seed coats held in this position. On a few such seedlings in large test tubes the fungus has been observed to fruit first on the uplifted seed coat and later to form typical reddish elongated lesions on the hypocotyl, presumably as the result of spores dropped from the seed coat above. Lesions have been produced on hypocotyls by rubbing conidia on them with an inoculating needle. On a few seedlings germinating in test tubes the mycelium of the frog-eye fungus has been observed to grow from the seed coat onto the cotyledonary tissue, but it has not exhibited any ability to parasitize these tissues to the extent of withdrawing nourishment from and sporulating on them. Reasoning from the behavior of the fungus on seedlings growing in large test tubes and from the fact that cotyledonary lesions have not been identified in the field, one is led to the assumption that in the field the fungus sporulates on the seed coat of infected seeds and the spores are then transported from there to the true leaves by the wind or other agencies. As the cotyledons are pushed upward through the soil by the elongating hypocotyl, the seed coat is pulled off the great proportion of the seedlings and left in the soil. Only a small proportion of the seed coats ever come above the ground and attain an elevation where spores formed on them are likely to be picked up by wind. This circumstance may account for the observed tardiness with which frog-eye arises and the slowness with which infestation becomes conspicuous when it originates from diseased seed. The writer has observed that field infestation reaches discernible proportions later in the summer when the disease arises from infected seed than when its source is in debris left on the field from a diseased crop. The percentage of infected seeds effective in initiating infections in the new crop is probably greatly reduced by the natural stripping off of the seed coat while still in the ground. This is a fortunate circumstance, for, otherwise, field infestations arising as they usually do from infected seed would reach harmful proportions earlier in the summer and thus result in greater harm to the plants.

SUMMARY

Infections by the frog-eye fungus (*Cercospora diazu* Miura) of soybean have been observed in large numbers not only on leaves, as noted in a previous publication, but also on stems, pods, and seeds.

The causal organism has been isolated from frog-eye lesions on leaves, stems, pods, and seeds, and reciprocal inoculations have been made. The cultures from these different sources were alike, and positive infections were obtained in sufficiently large numbers to justify the conclusion that the lesions on leaves, stems, pods, and seeds were caused by one and the same species, namely, the frog-eye fungus, *Cercospora diazu*.

Cauline lesions appear in abundance only in the late part of the growing season when stem tissues are ripening. The diseased areas are elongated and may be flattened or slightly sunken. The lesion is some shade of red when young, but changes to brown, then smoke gray with age. Black stromata of pin-point dimensions are often visible in large numbers on old lesions, and the diseased area may become black because of more or less pronounced stromatic development of dark-colored mycelium in the invaded tissue.

In stem lesions, the mycelium of the parasite grows chiefly in the cortex, being confined there by the presence of a pericyclic layer of sclerenchyma between cortical and phloem tissues. The phloem and cambium are usually damaged, however. Sometimes this is caused by actual invasion of these tissues by the fungus, but more often no mycelium is present in them, the obvious injury being due presumably to diffusion of toxic materials from the necrotic portion of the adjacent cortex.

Pod lesions are usually quite round, somewhat sunken, and vary in diameter from 1 to 4 mm. Young lesions on green pods are brown, often with a tint of red. A uniform brown color may persist as the pod matures, but usually on dry, ripe pods the diseased area appears as a light-brown spot encircled by a dark-brown ring. Conidiophores and conidia are often present.

Mycelium grows completely through the pod wall entering the thin white membrane which lines the pod and closely invests the seeds. Portions of this infected membrane often adhere to the seed coat and thus serve, but not invariably, to designate the individual seeds which are infected.

No definite lesion or marked discoloration is produced on the portion of the seed coat which develops in contact with a pod-wall lesion. However, there is often at this place a slight depression in the seed coat and a lack of the smoothness and luster characteristic of the coats of healthy seeds. This roughened, depressed area is a less conspicuous but more infallible sign of seed infection than is the presence of bits of pod-wall lining adhering to the seed coat.

Seed-treatment tests show that the frog-eye fungus makes intimate contact with a large proportion of the seeds which develop in touch with pod-wall lesions. In a large percentage of such seeds the fungus is so superficial as to be killed by seed disinfectants. It is actually in the seed-coat tissues and beyond the reach of disinfectants in a relatively few seeds. Infection of the embryo occurs rarely, if at all.

It is possible by inspection of a given seed lot to determine whether or not any considerable proportion of the pods from which the seed came were infected. Experience indicates that where as many as 10 percent of the seeds show signs of the frog-eye disease, practically 100 percent of the leaves of the plants which produced the seed were infected to the extent that considerable defoliation occurred.

The frog-eye fungus has been found still viable in potato-agar test-tube cultures 519 days old.

This fungus overwinters on diseased leaves and stems. The disease was initiated experimentally in an uninfested test plot by scattering diseased leaves over the ground. It has been observed to develop when disease-free seed was sown on land that bore a diseased crop the previous season. Breaking the infested stubble in the fall did not prevent or even appreciably retard disease development the next season. Diseased stems cannot be turned under sufficiently well to prevent recurrence of the disease if soybeans follow soybeans without an intervening crop.

The use of diseased seed is a means of introducing the disease into new fields and new communities.

VARIETAL DIFFERENCES IN COTTON BOLL SHEDDING AS CORRELATED WITH OSMOTIC PRESSURE OF EXPRESSED TISSUE FLUIDS[1]

By R. S. Hawkins, *agronomist*, S. P. Clark, formerly *assistant agronomist*, Geo. H. Serviss, formerly *research assistant in agronomy*, and Chas. A. Hobart, *research assistant in agronomy, Arizona Agricultural Experiment Station*

INTRODUCTION

Results previously reported[2] indicate an inverse relationship between the shedding of young cotton bolls of the Acala variety and the osmotic pressure of the expressed leaf fluids. Low osmotic pressures were found to be followed by high shedding and relatively high osmotic pressures by low shedding. Anatomical studies of stem tissues indicated that during periods of low osmotic pressure plant food was used largely to build vegetative tissues to the detriment of fruiting parts. Conversely, when osmotic pressures were relatively high vegetative growth was depressed and fruiting parts were supplied with adequate plant food, resulting in low shedding. Extremely high osmotic pressures induced by severe drought were followed not only by suppressed vegetative growth but also by intense shedding.

The findings previously reported with the Acala variety have been corroborated and additional data obtained on other cotton varieties, boll shedding in which does not always respond to osmotic-pressure variations in quite the same manner as in the Acala variety. Varietal differences in boll shedding have been investigated with the idea that differences between the osmotic pressures in the leaves and in the adjoining bolls of the same variety might account for heritable differences in shedding. If the osmotic pressures were materially higher in the leaves than in the adjoining bolls the bolls would not always be able to compete with the leaves for the water in the tissue fluids, and high shedding would follow. It was thought that the differences in osmotic pressure between leaves and bolls in the Pima variety of cotton, in which shedding is very low, seldom reaching more than 25 or 30 percent, might be found to be very small as compared with those in Acala, for example, in which boll shedding often reaches 70 or 80 percent.

METHODS

In the first experiment a comparison was made between the Pima and Acala varieties in the matter of boll shedding as affected by osmotic-pressure differences in the tissue fluids of the leaves and bolls. The plants were grown in 1926 on adjacent plots at the Salt River Valley Experiment Farm and given similar treatment through-

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[1] Received for publication Sept. 20, 1933; issued March, 1934.

[2] Hawkins, R. S., Matlock, R. L., and Hobart, C. PHYSIOLOGICAL FACTORS AFFECTING THE FRUITING OF COTTON WITH SPECIAL REFERENCE TO BOLL SHEDDING. Ariz. Agr. Expt. Sta. Tech. Bull. 46, pp. 361–407, illus. 1933.

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Jan. 15, 1934
Key no. Ariz.-9

out the season. The soil is a fertile clay loam, and excellent plant growth was obtained with both varieties. Climatological measurements, including atmospheric and soil temperature, rainfall, cloudiness, wind movement, and evaporation, were obtained in connection with another experiment on water variations in the leaves of these two varieties of cotton. These data, together with soil-moisture data, are recorded elsewhere[3] and will not be repeated here since this experiment is concerned primarily with differences in shedding as influenced by variations in osmotic pressure and only incidentally with the factors that affect changes in osmotic pressure.

The shedding behavior of the 2 varieties was ascertained by daily tagging all flowers on 25 adjacent plants in each plot and then noting the number of tagged bolls that were shed each day. Leaves and day-old bolls were collected daily at 8 a.m. from July 28 to August 25 for the determinations of osmotic pressure. The leaves used were those adjacent to the bolls collected. Two men made the collections; one collected material from the Acala variety while the other made parallel collections from the Pima variety. Seven tubes of leaves and seven tubes of bolls were collected each day from each variety. The tubes were placed in an ice-salt freezing mixture within a few minutes after the daily collections were completed. After several hours of freezing, the material while still in the tubes was removed from the freezing mixture and allowed to thaw for a short time. The juices were then expressed by means of a fruit press. Harris[4] employed a similar press in his work with cotton when a press equipped with a pressure gage was not available, a procedure not altogether desirable but at times unavoidable.

The freezing-point method of determining the atmospheres of osmotic pressure, as described by Dixon and Atkins,[5] Gortner and Harris,[6] and Lawrence and Harris[7] was used.

In 1927 a second experiment was made. In this experiment shedding and osmotic-pressure variations were investigated with four varieties of cotton, viz: Pima, Acala, an F_1 cross between Acala and Pima, and a small-boll American Indian variety known as Sacaton Aboriginal. These four varieties exhibit wide variations in shedding behavior. The plots were located on the Salt River Valley Experiment Farm and soil conditions other than moisture were approximately similar to those in experiment 1. The technic for osmotic-pressure determinations was not varied from that employed in experiment 1 except that 6 tubes of leaves and 6 tubes of bolls of each variety were used for the daily determinations.

RESULTS AND DISCUSSION

EXPERIMENT 1

The shedding behavior of the Acala variety was quite consistently correlated with osmotic pressures in the leaves and bolls, being high when osmotic values were low, and low when osmotic values were high (fig. 1). These results are in accord with those reported previously.[8] Two irrigations were given during the portion of the fruiting period under investigation, that is, on August 4 and 19. These dates coincide with the two peaks in the osmotic-pressure variations. Thus the irrigations indirectly influenced the shedding behavior of the plants.

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[3] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

[4] HARRIS, J. A., LAWRENCE, Z. W., HOFFMAN, W. F., LAWRENCE, J. V., and VALENTINE, A. T. THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F_1 HYBRID. Jour. Agr. Research 27: 267–328, illus. 1924.

[5] DIXON, H. H., and ATKINS, W. R. G. OSMOTIC PRESSURES IN PLANTS. METHODS OF EXTRACTING SAPS FROM PLANT ORGANS. Roy. Dublin Soc. Sci. Proc. 13: 422–438. 1913.

[6] GORTNER, R. A., and HARRIS, J. A. NOTES ON THE TECHNIQUE OF THE DETERMINATION OF THE DEPRESSION OF THE FREEZING POINT OF VEGETABLE SAPS. Plant World 17: 49–53. 1914.

[7] LAWRENCE, J. V., and HARRIS, J. A., THE EXTRACTION OF SAP FROM PLANT TISSUES BY PRESSURE. Biochem. Bull. 5: 139–142, illus. 1916.

In the Acala variety the osmotic pressures in the bolls were always somewhat lower than in the leaves. The same was true of the Pima variety (fig. 2) with the exception of the material collected on August 18 and 19, when the osmotic pressures in the bolls were slightly higher than in the leaves. However, the results do not appear to substantiate the assumption that wide differences between osmotic pressures in the leaves and bolls on the same plants at any given date materially increase shedding. For example, shedding was at a minimum with bolls whose flowers opened on August 4, although osmotic pressures in the bolls and leaves were at wider variance than at other times when shedding was higher (fig. 1). Many similar examples which refute the foregoing assumption are evident from a study of the shedding and osmotic-pressure curves in figures 1, 2, 4, and 5.

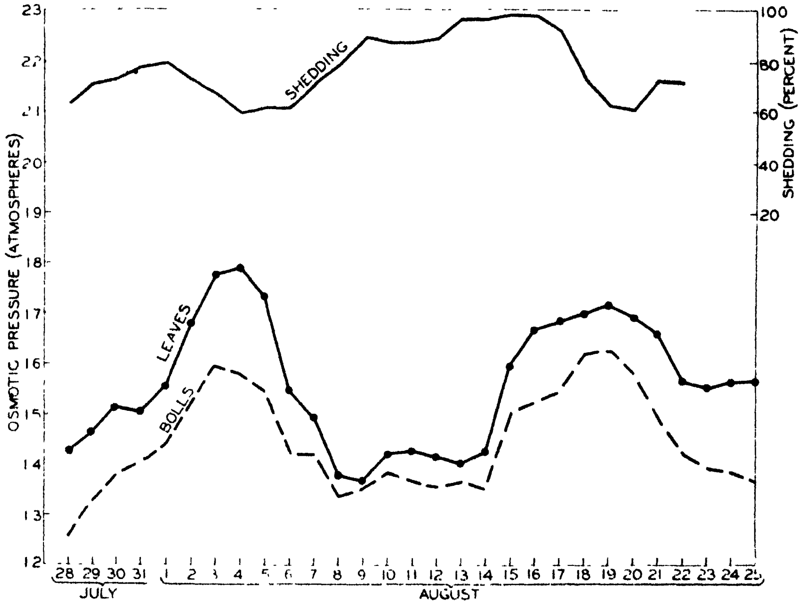

FIGURE 1. Shedding percentages and osmotic pressures in the leaves and bolls of Acala cotton.

The curves for Pima, presented in figure 2, show little if any correlation between shedding and osmotic pressure. Shedding is very low in this variety, however, seldom reaching more than 30 percent on any one day. In this investigation the bolls shed by the Pima plants averaged between 17 and 18 percent. Most of the

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[8] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

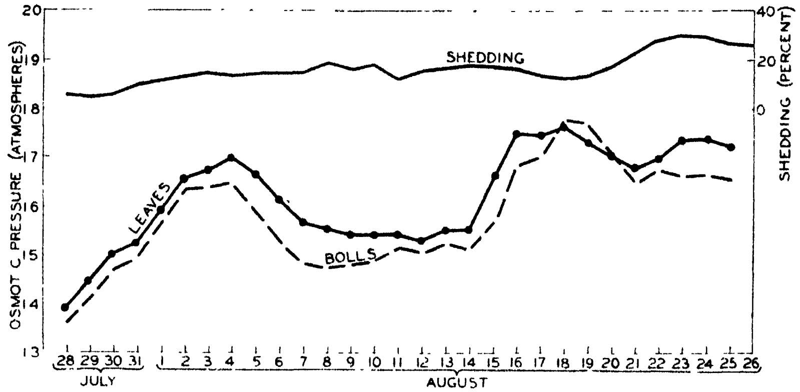

FIGURE 2.—Shedding percentages and osmotic pressures in the leaves and bolls of Pima cotton.

shedding of the Pima bolls may have been due to insect damage or to a lack of effective pollination, or both. With a heavy-shedding variety like Acala, which shed 78 percent during this same period, the same amount of insect damage or imperfect pollination would be greatly overshadowed by other influential factors such as asmotic pressure of the tissue fluids.

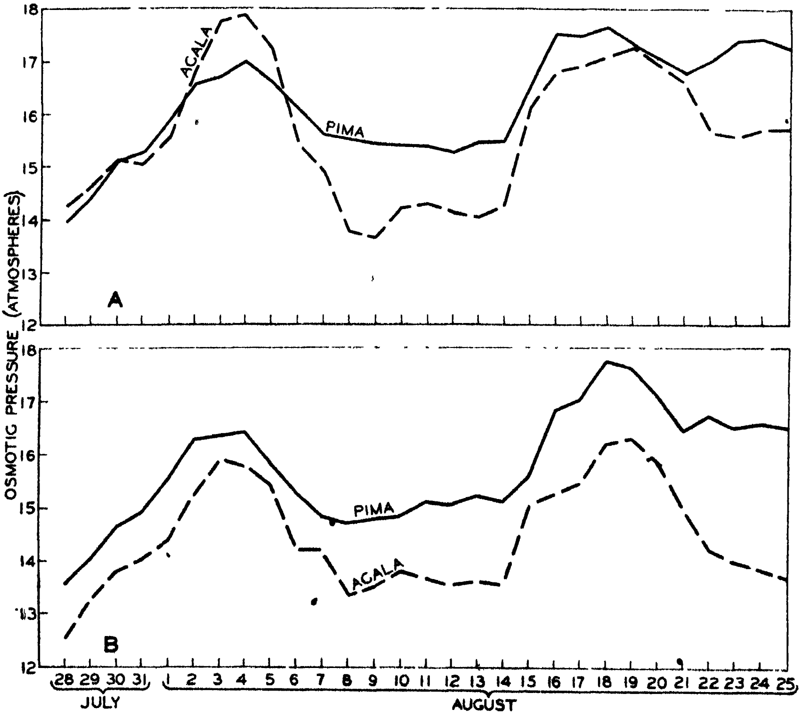

FIGURE 3.—Osmotic pressure in the leaves (*A*) and bolls (*B*) of Pima and Acala cottons.

It has been shown previously[9] that high osmotic pressures are correlated with decreased shedding in the Acala variety. The osmotic pressures were always materially higher in the Pima bolls than in the Acala bolls (fig. 3, *B*). A similar situation prevailed in the leaves of the two varieties with few exceptions (fig. 3, *A*). These higher osmotic values in the Pima plants may account for the limited amount of boll shedding. This assumption is supported further by data obtained in connection with experiment 2.

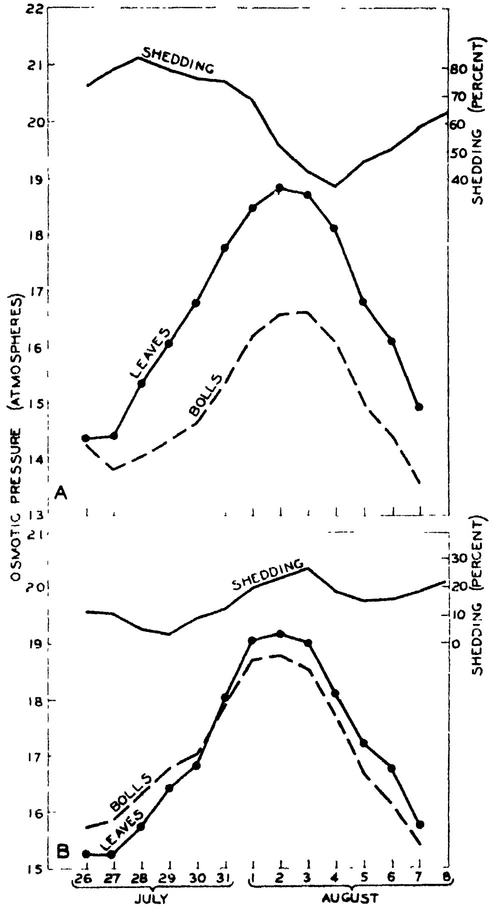

FIGURE 4.—Shedding percentages and osmotic pressures in the leaves and bolls of (*A*) Acala and (*B*) Pima cottons.

EXPERIMENT 2

Osmotic pressures and boll shedding in the Acala variety again were correlated (fig. 4, *A*). The characteristic wide spread between the osmotic pressures in the leaves and in the bolls was also exhibited. Whether this wide spread has any significance in relation to shedding is not known. No correlation between osmotic pressures and shedding is indicated in the Pima variety, which coincides with the results obtained in experiment 1 (fig. 4, *B*). A good correlation is shown for the first-generation hybrid of Pima and Acala at the period of high osmotic pressure and immediately following this peak, although little if any is shown for the rest of the period under investigation (fig. 5, *A*). The osmotic pressure values for the bolls of the Pima×Acala hybrid were intermediate between those for the bolls of the two parents, and the correlaton between osmotic pressure and shedding in the hybrid conforms to expectation. The Sacaton Aboriginal, a variety which flowers profusely, had consistently low osmotic values. Variations in shedding were not great and the shedding curve shows a good negative correlation with osmotic pressure (fig. 5, *B*).

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[9] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

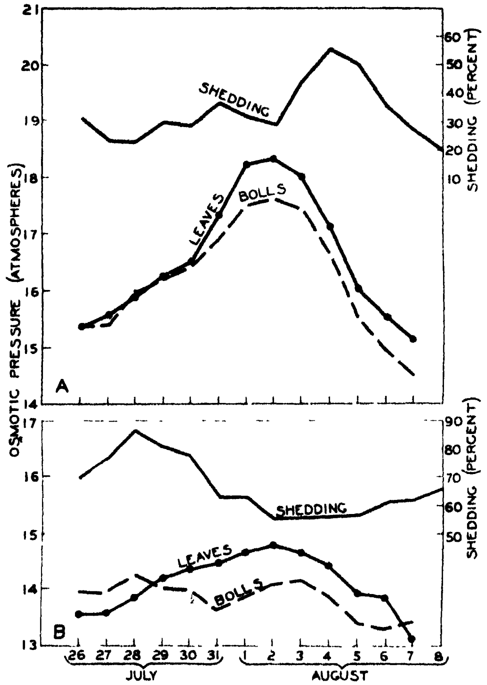

FIGURE 5.—Shedding percentages and osmotic pressures in the leaves and bolls of (*A*) an F_1 Pima × Acala hybrid and (*B*) of Sacaton Aboriginal cotton.

The shedding curves of the four varieties have been superimposed in figure 6. The superimposed curves showing the osmotic pressures of the bolls and of the leaves are shown in figure 7. A comparison of the graphs demonstrates that the order of arrangement of the four varieties on the basis of shedding behavior is the reverse of their arrangement on the basis of osmotic pressure in the bolls. Pima had the lowest shedding and the highest osmotic pressures, while Sacaton Aboriginal had the highest shedding and the lowest osmotic pressures. The other two varieties were intermediate both in shedding and in osmotic pressure. These results are entirely in accord with those obtained in experiment 1 and with the data reported previously.[10] The osmotic pressures in the leaves of

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[10] HAWKINS, R. S., MATLOCK, R. L., and HOBART, C. (See footnote 2.)

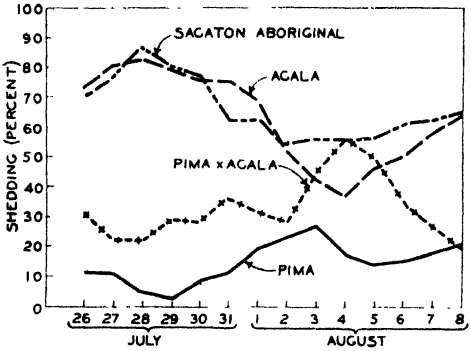

FIGURE 6.—Shedding percentages of four varieties of cotton.

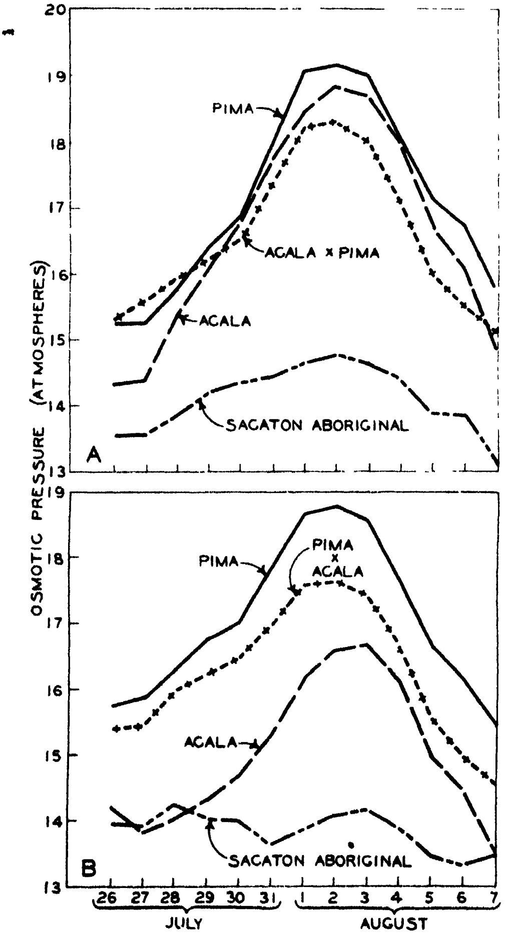

FIGURE 7.—Osmotic pressures in the leaves (*A*) and bolls (*B*) of four varieties of cotton.

Pima are the highest and those in the leaves of Sacaton Aboriginal the lowest, but the order is reversed with the two intermediate varieties. Possibly this indicates that osmotic pressures in the bolls are more directly correlated with shedding behavior than are the osmotic values in the leaves, although both undoubtedly are involved.

SUMMARY

The shedding of young Acala cotton bolls was definitely correlated with the osmotic pressures of the expressed tissue fluids of the leaves and day-old bolls.

The daily changes in boll shedding and osmotic pressures show no correlation in the Pima variety. The extremely low shedding coupled with comparatively high osmotic pressures may account for the lack of correlation.

The spread between the osmotic pressures in the leaves and in the bolls does not seem to be correlated with shedding.

The superimposed shedding curves of four varieties differing widely in shedding behavior show a reverse arrangement as compared with the superimposed curves showing osmotic pressures in the bolls, which indicates a good negative correlation between shedding and osmotic pressure in the bolls.

Boll shedding is somewhat more closely correlated with osmotic pressure in the bolls than with osmotic pressure in the leaves.

BORDER EFFECT IN IRRIGATED PLOTS OF MARQUIS WHEAT RECEIVING WATER AT DIFFERENT TIMES[1]

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INTRODUCTION

Extensive data are available on the border effect in plots in which different varieties of plants have been grown under humid conditions. Little study has been made, however, of the border effect on the same crops grown under irrigation. This paper presents the results of experiments made to determine the border effect on Marquis wheat grown in plots irrigated at different stages, and shows the relationship of the yields when various numbers of border rows are included.

MATERIALS AND METHODS

The plots were located on land that had been fallowed the previous season. The wheat was sown with a 16-disk drill, and after sowing plots approximately one five-hundredth of an acre were measured off and diked.

The plots were laid out end to end, a dike about 1 foot wide separating each plot. In the fall one plot was diked and irrigated with 6 inches of water. The basin method of irrigation was used.[2] After the soil had dried on the surface and before snow had fallen, the dikes were leveled with the rest of the series. This prevented any unequal drifting of snow over the area used. In the spring after planting, a second plot received 7 inches of water at germination, and the other plots received 1 inch of water to insure an even germination. The third plot received a 6-inch irrigation at jointing, or just before heading. All of the plots, therefore, received a total irrigation of 7 inches as well as the seasonal rainfall. There were three plots for each treatment each year. At harvest time 1 foot was cut from the end of each row, leaving a plot 10 feet long. The plots were harvested as follows: Outside border rows 1 and 16 were harvested separately with grass shears; rows 2 and 15 and 3 and 14 were similarly harvested; rows 4 to 13, inclusive, were harvested together, making the 10 center rows. All of the bundles were wrapped in cloth and cured under cover. A modified Cornell thresher was used for threshing all bundles. Total weights of grain and straw, uncleaned weight of grain, and cleaned weight of grain were taken. The difference between the cleaned weight and the total weight is considered as straw yield. The plots were systematically distributed.

The experiment as outlined was carried on for 4 years, 1928 to 1931, inclusive.

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[1] Received for publication Aug. 2, 1933; issued March, 1934. Contribution from the Department of Agronomy, Colorado Agricultural Experiment Station.

[2] KEZER, A., and ROBERTSON, D. W. THE CRITICAL PERIOD OF APPLYING IRRIGATION WATER TO WHEAT. Jour. Amer. Soc. Agron 19. 93. 1927.

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RAINFALL

Rainfall, which varied during the different seasons, affected the total yield. In the dry season of 1931 the yield for all plots was smaller than in any other year of the experiment.

The rainfall data for 1928 to 1931 are given in table 1.

TABLE 1.—*Rainfall* [a] *at Fort Collins, Colo., 1928–31*

Month	1928	1929	1930	1931	Month	1928	1929	1930	1931
	Inches	*Inches*	*Inches*	*Inches*		*Inches*	*Inches*	*Inches*	*Inches*
January	0.26	0.21	0.45	0.00	August	.69	2.35	5.36	.75
February	.52	.70	.70	1.26	September	.09	2.13	.16	.51
March	1.38	1.78	.56	.41	October	1.50	.99	.35	1.00
April	1.02	2.37	.49	1.07	November	1.15	.93	.70	.63
May	3.01	.92	3.92	2.93	December	.06	.09	.14	.18
June	2.95	.64	1.50	1.46					
July	.79	.46	1.04	.05	Total	13.42	13.57	15.37	10.25

[a] The rainfall records for January, February, March, November, and December were obtained from R. E. Trimble, assistant irrigation investigator.

DATES OF PLANTING, IRRIGATION, AND RIPENING

Marquis wheat was planted on April 9, 1928; April 8, 1929; April 7, 1930; and April 6, 1931. The dates of irrigation and the dates of ripening of the grain on the various plots are given in table 2. This table shows little, if any, difference in the date of maturity of the grain, in any one year, on the plots treated.

TABLE 2.—*Dates of applying irrigation water to plots, and of ripening of the grain on the variously treated plots, 1928–31*

DATES OF APPLYING IRRIGATION WATER TO PLOTS

Treatment	Data for plots planted on—			
	Apr 9, 1928	Apr 8, 1929	Apr 7, 1930	Apr 6, 1931
Irrigation in fall	Sept 9, 1927	Sept. 10, 1928	Oct. 1, 1929	Oct. 6, 1930
Irrigation at germination	Apr. 14, 1928	Apr. 20, 1929	Apr 14, 1930	Apr 13, 1931
Irrigation at jointing	June 7, 1928	June 18, 1929	June 10, 1930	June 15, 1931

DATES OF RIPENING OF THE GRAIN

Irrigation in fall	Aug. 8, 1928	July 30, 1929	July 22, 1930	July 24, 1931
Irrigation at germination	do	do	do	Do
Irrigation at jointing	do	do	do	July 26, 1931

YIELD DATA

In studying the border effect, 10 center rows are considered as a unit of 100 percent. The 12 rows are made up of the 10 center rows and the inside border rows (3 and 14). The 14-row plots are made up of the 10 center rows, the inside border rows, and the middle border rows (2 and 15). The 16-row plots include the 10 center rows, the inside border rows, the middle border rows, and the outside rows (1 and 16). Plots of 10, 12, 14, and 16 rows are used in the discussion of border effect. The arrangement of the rows is shown in figure 1.

The yield data show considerable variation between the different years. When the yields of the outside rows, 1 and 16, are compared with those of the 10 center rows, a considerably higher percentage value is obtained than when the yield of the entire plot (16 rows) is compared with the yield of the 10 center rows. The same is true of rows 2 and 15, but to a lesser degree. The average yield for each plot of 10, 12, 14, and 16 rows is given in table 3. The table shows that while the yield varied in different years the yield in percentage

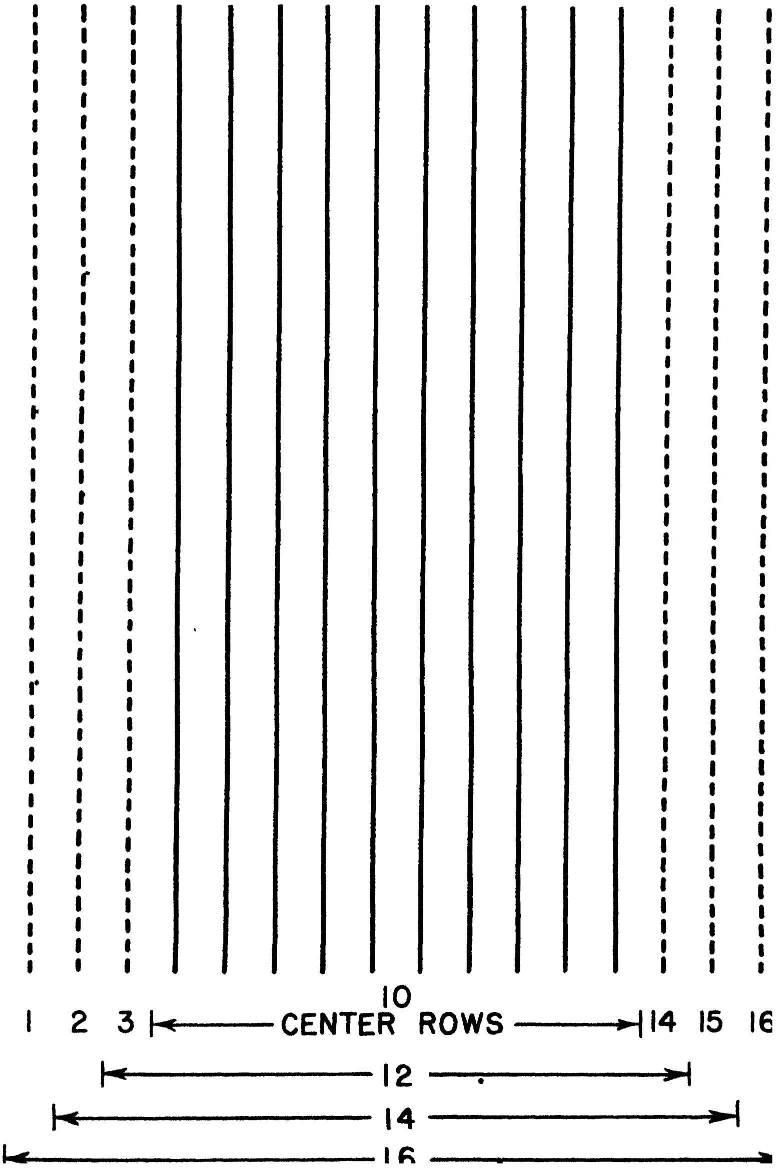

FIGURE 1.—The arrangement of rows in the differently treated plots used in the computation of yield data.

when compared with the fall-irrigated plots as 100 percent holds the same relative position for plots of 10, 12, 14, or 16 rows.

The variation in straw yield was about the same as in grain yield, except that yields of straw were slightly smaller for the plots irrigated at germination and slightly larger for those irrigated at jointing.

Table 3.—*Yields from different numbers of border rows harvested from plots of Marquis wheat receving irrigations at different times, 1928–31*

Year	Grain yield from plots irrigated at time indicated						Straw yield from plots irrigated at time indicated					
	Fall		Germination		Jointing		Fall		Germination		Jointing	
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
						10 CENTER ROWS						
1928	1,384	100	1,527	110	1,533	111	2,892	100	2,785	96	3,433	119
1929	1,020	100	884	87	1,434	141	1,667	100	1,459	88	2,470	148
1930	1,180	100	1,144	97	1,390	118	2,290	100	2,004	88	3,299	144
1931	825	100	757	92	1,111	135	1,447	100	1,211	84	2,075	143
Average	1,102	100	1,078	98	1,367	124	2,074	100	1,865	90	2,819	135
						12 ROWS						
1928	1,647	100	1,839	112	1,815	110	3,408	100	3,330	98	4,075	120
1929	1,226	100	1,090	89	1,715	140	2,002	100	1,752	88	2,992	149
1930	1,389	100	1,350	97	1,674	121	2,714	100	2,372	87	3,942	145
1931	994	100	910	92	1,348	136	1,733	100	1,489	86	2,539	147
Average	1,314	100	1,207	99	1,638	125	2,461	100	2,236	91	3,387	137
						14 ROWS						
1928	2,023	100	2,238	111	2,229	110	4,154	100	4,084	98	4,955	119
1929	1,535	100	1,383	90	2,114	138	2,501	100	2,214	88	3,695	148
1930	1,718	100	1,618	94	2,063	120	3,337	100	2,844	85	4,798	144
1931	1,191	100	1,097	92	1,596	134	2,107	100	1,781	85	3,009	143
Average	1,617	100	1,584	98	2,001	124	3,025	100	2,731	90	4,111	136
						16 ROWS						
1928	2,551	100	2,900	114	2,870	113	5,237	100	5,212	100	6,287	120
1929	2,007	100	1,831	91	2,707	135	3,253	100	2,958	91	4,664	143
1930	2,210	100	2,006	91	2,555	116	4,213	100	3,521	84	5,768	137
1931	1,387	100	1,336	96	1,861	134	2,572	100	2,302	90	3,604	140
Average	2,039	100	2,018	99	2,498	123	3,819	100	3,499	92	5,081	133

By comparing the yield of the 10 center rows alone with the 10 center rows plus the inside border rows (12 rows), the 10 center rows plus the inside and middle border rows (14 rows), and the entire plot of 16 rows, the increase in percentage yield over the 10 center rows is found to be gradual and is approximately the same for all treatments. While the yearly yield of grain varies from 825 to 1,384 g, the percentage increase for the different number of border rows is approximately the same. However, the effect of the outside border rows in the dry season of 1931 is slightly less than in the other 3 years. It varies only 6 percent between treatments. Table 4 gives the average yearly yield for the 10 center rows and for the 10 center rows plus 2, 4, and 6 border rows.

The increase in yield of both straw and grain is gradual. The average figures for grain yield range from 119 to 120 percent for the 10 center rows plus the inside border row, 146 to 147 for the 10 center rows plus the inside and middle border rows, and from 183 to 178 percent for the entire plot (fig. 2). A very similar range is found for the straw yield. The time of treatment had no consistent effect on the yield, all treatments having about the same percentage increase for the different number of border rows.

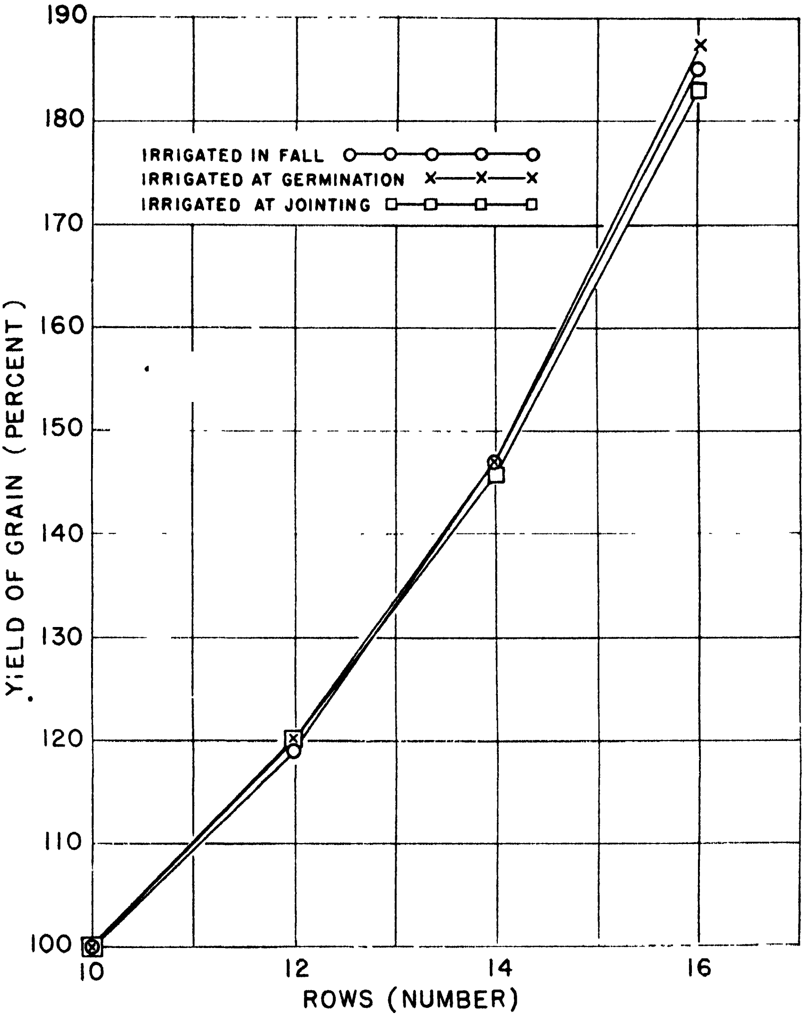

FIGURE 2.—Percentage yield of grain for plots of 10, 12, 14, and 16 rows.

TABLE 4.—*Yields from 10 center rows and from plots consisting of 10 center rows plus 2, 4, or 6 border rows, 1928–31*

Year	Rows	Grain yield from plots irrigated at time indicated						Straw yield from plots irrigated at time indicated					
		Fall		Germination		Jointing		Fall		Germination		Jointing	
	Number	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
1928	10	1,384	100	1,527	100	1,533	100	2,892	100	2,785	100	3,433	100
	12	1,647	119	1,839	120	1,815	118	3,408	118	3,330	120	4,075	119
	14	2,023	146	2,238	147	2,229	145	4,154	144	4,084	147	4,955	144
	16	2,551	184	2,900	190	2,870	187	5,237	181	5,212	187	6,287	183
1929	10	1,020	100	884	100	1,434	100	1,667	100	1,459	100	2,470	100
	12	1,226	120	1,090	123	1,715	120	2,002	120	1,752	120	2,992	121
	14	1,535	150	1,383	156	2,114	147	2,501	150	2,214	152	3,695	150
	16	2,007	197	1,831	207	2,707	189	3,253	195	2,958	202	4,664	189
1930	10	1,180	100	1,144	100	1,390	100	2,290	100	2,004	100	3,299	100
	12	1,389	118	1,350	118	1,674	120	2,714	119	2,372	118	3,942	119
	14	1,718	146	1,618	141	2,063	148	3,337	146	2,844	142	4,798	145
	16	2,210	187	2,006	175	2,555	184	4,213	184	3,524	176	5,768	175
1931	10	825	100	757	100	1,111	100	1,447	100	1,211	100	2,075	100
	12	994	120	910	120	1,348	121	1,733	120	1,489	123	2,539	122
	14	1,191	144	1,097	145	1,596	144	2,107	146	1,781	147	3,009	145
	16	1,387	168	1,336	176	1,861	168	2,572	178	2,302	190	3,604	174
Average	10	1,102	100	1,078	100	1,367	100	2,074	100	1,865	100	2,819	100
	12	1,314	119	1,297	120	1,638	120	2,464	119	2,236	120	3,387	120
	14	1,617	147	1,584	147	2,000	146	3,025	146	2,731	146	4,114	146
	16	2,039	185	2,018	187	2,498	183	3,819	184	3,499	187	5,081	180

There is no consistent increase or decrease in variability of the plots. Table 5 gives the average yield in grams of the fall-irrigated plots and the probable error in percentage of the mean. The probable errors were determined by the deviation-from-the-mean method. There is a variation between different years, but no consistent trend up or down as the number of rows increase.

TABLE 5.—*Comparison of variability in the different fall-irrigated plots as determined by the probable error of the mean, in percentage*

Year	Rows	Grain yield		Straw yield	
		Fall-irrigated plots	Probable error	Fall-irrigated plots	Probable error
	Number	*Grams*	*Percent*	*Grams*	*Percent*
1928	10	1,384	±2.74	2,892	±3.47
	12	1,647	±2.50	3,408	±3.46
	14	2,023	±2.22	4,154	±3.32
	16	2,551	±2.87	5,237	±3.59
1929	10	1,020	±2.28	1,667	±2.56
	12	1,226	±2.24	2,002	±3.42
	14	1,535	±1.82	2,501	±2.96
	16	2,007	±1.60	3,253	±2.79
1930	10	1,180	±2.25	2,290	±2.69
	12	1,389	±2.17	2,714	±2.52
	14	1,718	±1.87	3,337	±2.28
	16	2,210	±1.27	4,213	±1.64
1931	10	825	±1.66	1,447	±1.80
	12	994	±1.95	1,733	±2.40
	14	1,191	±1.92	2,107	±2.22
	16	1,387	±2.32	2,572	±2.05

COVERED PLOTS

A study was made of the yields from covered plots. Except for the fact that covers were placed over the plots to prevent additional moisture from falling on them, the treatments in these experiments were similar to those employed in the study of border effect on open plots. For a detailed description of the covers, see Kezer and Robertson.[3] The plots received only 7 inches of water from the time of planting to harvest. These experiments were conducted from 1929 to 1931. In 1929 and 1930 six replications were used, and in 1931 three. In diking the plots to facilitate irrigation, the outside rows were more or less covered. The effect of the partially developed outside rows on the middle border rows under cover was determined by correlating the grain yield of row 1 plus row 16 and row 2 plus row 15.

The following correlations were found: -0.2548 ± 0.0997 in 1929; $+0.0109 \pm 0.0938$ in 1930; $+0.3234 \pm 0.1036$ in 1931. All of the correlations were small and indicate very little relationship between the yield of the outside and middle border rows. In determining the correlations only 18 plots were used. In discussing the border effect of the covered plots, rows 1 and 16 are not used, since only fragmentary stands in these rows were obtained.

The data from the covered plots are given in table 6. The effect of eliminating the influence of rainfall by covering the plots during rainstorms and at night is shown by the slightly increased yield ratio in the plots irrigated at jointing. The border effect remains the same for 12- or 14-row plots in different years. These results are similar to those obtained with the uncovered plots. The straw yield and total yield of grain and straw show similar results.

A comparison of the percentage increase of the 12- and 14-row plots over the 10 center rows (table 6) indicates a slightly greater increase in yield of grain in 1931 for the 12- and 14-row plots. However, the increase was similar for all treatments. These results again indicate that there is no difference in comparative yields of the different-sized plots for the different treatments. The same is true for the straw yield.

TABLE 6.—*Comparison of yield of 10 center rows and of 10 center rows plus 2 or 4 border rows, covered plots, 1929–31*

Year	Rows	Grain yield					
		Fall		Germination		Jointing	
	Number	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
1929	10	501	100	438	100	564	100
	12	621	124	539	123	690	122
	14	797	159	698	159	885	157
1930	10	386	100	399	100	464	100
	12	490	127	500	125	587	127
	14	651	169	667	167	795	171
1931	10	147	100	183	100	202	100
	12	196	133	240	131	265	131
	14	271	184	317	173	325	161
Average	10	345	100	340	100	410	100
	12	436	126	426	125	514	125
	14	573	166	561	165	668	163

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[3] KEZER, A., and ROBERTSON, D. W. See footnote 2; pp. 80–116.

The probable error is greater for the covered plots (table 7) than for the open plots. In 2 years out of 3 the error decreased as the number of rows increased. In 1929 both the grain-yield and the straw-yield error was smallest for the 10-row plot. The probable error, however, varied more between years than it did between plots. These data indicate that Marquis wheat receiving irrigations at different times gives comparable yields as accurately from plots consisting of 10 center rows plus 4 border rows as from the 10 center rows alone.

Table 7.—*Probable error in percentage of mean for plots of 10 rows, 10 rows plus 2 border rows, and 10 rows plus 4 border rows; covered plots, irrigated in the fall, 1929–31*

Year	Rows	Grain yield	Probable error	Straw yield	Probable error
	Number	*Grams*	*Percent*	*Grams*	*Percent*
1929	10	501	±2.44	1,037	±1.52
	12	621	±2.51	1,277	±1.71
	14	797	±2.47	1,610	±1.76
1930	10	386	±4.56	860	±2.89
	12	490	±4.20	1,091	±2.76
	14	651	±3.76	1,455	±2.54
1931	10	147	±7.69	448	±2.90
	12	196	±6.78	558	±2.49
	14	271	±5.20	724	±2.30

STATISTICAL ANALYSES

A summary of the yields in relation to their probable errors is given in table 8.

TABLE 8.—*Total and average yields of grain from covered plots consisting of 10 center rows plus 0, 2, 4, and 6 border rows*

Year	Time of irrigation	Data for yield in grams per plot of number of rows indicated							
		10 rows		12 rows		14 rows		16 rows	
			D/PE		D/PE		D/PE		D/PE
1928	In fall	1,384±38.00		1,647±41.00		2,023±45.00		2,551± 73.00	
	At germination	1,527±42.00		1,839±46.00		2,238±50.00		2,900± 83.00	
	At jointing	1,533±42.00		1,815±45.00		2,229±49.00		2,870± 82.00	
	Difference								
	Germination-fall	143±56.64	2.52	192±61.62	3.12	215±67.27	3.20	349±110.53	3.16
	Jointing-fall	149±56.64	2.63	168±60.87	2.76	206±66.53	3.10	319±109.77	2.91
1929	In fall	1,020±23.00		1,226±27.00		1,535±28.00		2,007± 32.00	
	At germination	884±20.00		1,090±24.00		1,383±25.00		1,831± 29.00	
	At jointing	1,434±33.00		1,715±38.00		2,114±38.00		2,707± 43.00	
	Difference								
	Germination-fall	−136±30.48	4.46	−136±36.12	3.77	−152±37.54	4.05	−176± 43.19	4.07
	Jointing-fall	414±40.22	10.29	489±46.62	10.49	579±47.20	12.26	700± 53.60	13.06
1930	In fall	1,180±27.00		1,389±30.00		1,718±32.00		2,210± 28.00	
	At germination	1,144±26.00		1,350±29.00		1,618±30.00		2,006± 25.00	
	At jointing	1,390±31.00		1,674±36.00		2,063±39.00		2,555± 32.00	
	Difference								
	Germination-fall	−36±37.50	.96	−39±41.72	.93	−100±43.86	2.28	−204± 37.54	5.43
	Jointing-fall	210±41.11	5.11	285±46.86	6.08	345±50.45	6.84	345± 42.52	8.11
1931	In fall	825±14.00		994±19.00		1,191±23.00		1,387± 32.00	
	At germination	757±13.00		910±18.00		1,097±21.00		1,336± 31.00	
	At jointing	1,111±18.00		1,348±26.00		1,596±31.00		1,861± 43.00	
	Difference								
	Germination-fall	−68±19.10	3.56	−84±26.17	3.21	−94±31.14	3.02	−51± 44.55	1.14
	Jointing-fall	286±22.80	12.54	354±32.20	10.99	405±38.60	10.49	474± 53.60	8.84
Average	In fall	1,102±12.00		1,314±15.00		1,617±16.00		2,039± 21.00	
	At germination	1,078±12.00		1,297±14.00		1,584±15.00		2,018± 21.00	
	At jointing	1,367±15.00		1,638±18.00		2,001±19.00		2,498± 26.00	
	Difference								
	Germination-fall	−24±16.97	1.41	−17±20.52	.83	−33±21.93	1.50	−21± 29.70	.71
	Jointing-fall	265±19.21	13.79	324±23.43	13.83	384±24.84	15.46	459± 33.42	13.73

The difference between the plots irrigated in the fall and those irrigated at germination is not significant, as is shown by the D/PE for the average yields. The degree of significance varies, however, in different years. For 1928 the deviation divided by the probable error of a difference is more than three for plots including 2, 4, and 6 border rows. In 1929 all of the differences are significant. In 1930 only the 16-row plots (10 center rows + 6 border rows) show a significant difference. In 1931 the 10-, 12-, and 14-row plots show a significant difference.

When the difference between the plots irrigated at jointing and those irrigated in the fall are considered, a significant difference is found only for the 14-row plot in 1928. However, in all of the other years there is a significant difference for all of the plots. There is no consistent increase, however. In 1928 there is a slight increase in the size of the difference divided by the probable error of a difference up to 14 rows, then there is a slight decrease. In 1929 and 1930 there is an increase up to the 16-row plots, but in 1931 there is a decrease, the 10-row plots having the greatest difference The data for the average yields show no general trend in the size of the difference. The average results when interpreted in relation to their probable errors show a significant difference between the plots irrigated in the fall and those irrigated at jointing for all combinations of the center 10 rows with the various number of border rows. The difference between the fall-irrigated plots and the plots irrigated at germination is not significant in any of the comparisons made on average yields. These results again indicate that comparable results can be obtained from irrigated plots whether the border is removed or whether it is included in the yields.

Similar results were obtained with the covered plots.

SUMMARY

The effect of different numbers of border rows on the yield of plots of Marquis wheat receiving different irrigation treatments was studied.

The yield increased as the size of the plot increased, but the percentage increase was uniform for the three different treatments employed.

Although the average yearly yields varied, the border effect remained uniform with the exception of the outside rows (1 and 16) In 1931, which was a very dry year, less border effect was found from the outside rows.

The variation as determined by the percentage probable error did not increase or decrease for plots of different sizes. It varied in different years with the different number of border rows. The variation was similar for both grain and straw yields.

When the same amount of irrigation water was applied to Marquis wheat at different times, the comparable yields were the same for plots of 10 rows, and for 10 plus 2, 4, or 6 border rows.

The difference in the average yield between the plots irrigated in the fall and those irrigated at germination was not significant for plots of 10, 12, 14, or 16 rows; the difference in the average yield between plots irrigated in the fall and those irrigated at jointing was significant for plots of 10, 12, 14, or 16 rows.

THE INFLUENCE OF PHOSPHORUS DEFICIENCY IN DAIRY COWS ON THE COEFFICIENT OF DIGESTIBILITY AND THE BALANCE OF CALCIUM AND PHOSPHORUS[1]

By W. H. Riddell, *dairy production investigations, Department of Dairy Husbandry;* J. S. Hughes, *animal nutrition, Department of Chemistry;* and J. B. Fitch, *head of Department of Dairy Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

A frequent observation in cases of aphosphorosis in cattle is the general lack of thrift and the poor condition of the animals affected.[2] Theiler and his associates,[3] after extensive investigation of the problem in South Africa, concluded that phosphorus was a limiting factor in growth and voluntary food consumption and a dominating factor in the maintenance of live weight under ordinary conditions of veld grazing.

In a study of the energy requirement per pound of gain for dairy cows on a low phosphorus ration, Eckles and Gullickson [4] reported that a plane of feeding at least 120 percent of that specified by the feeding standards in general use was necessary to even maintain the live weight of the experimental animals.

It has been definitely established that there is a lowered feed utilization in the phosphorus-deficient animal. The question may be raised, therefore, as to whether the condition of aphosphorosis adversely affects the digestibility of the ration. The evidence bearing on the effect of a lowered mineral intake on digestion is limited. Evans [5] reported the results of a series of digestion trials with swine suffering from prolonged calcium deficiency. Twenty-two trials showed "no enhanced effect on the digestibility of the organic constituents of the food on adding calcium carbonate to a lime-deficient ration." Woodman and Evans [6], working with sheep, concluded that a deficiency of calcium and phosphorus in forage did not cause it to be digested any less efficiently.

These results would suggest that the prolonged feeding of a phosphorus-deficient ration in all probability would not materially influence digestion. However, since there appeared to be no published results of digestion trials for animals in advanced stages of aphosphorosis, it was planned to secure definite information on this subject and on the mineral metabolism of affected animals at the same time.

EXPERIMENTAL PROCEDURE

Late in October 1929, three animals were selected and placed on the low-phosphorus experimental ration: (1) Control cow, E-7 (re-

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[1] Received for publication July 24, 1933, issued March 1934. This paper represents a portion of a thesis presented by W. H Riddell in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Minnesota, 1932. Contribution no 88 of the Department of Dairy Husbandry, and no 174 of the Department of Chemistry.

[2] An excellent and comprehensive review of the literature pertaining to phosphorus deficiency in ruminants has appeared in the following publication Theiler, A., and Green, H H. APHOSPHOROSIS IN RUMINANTS. Nutrition Abs & Rev 1 359-385 1932

[3] Theiler, A, Green, H H., and duToit, P. J PHOSPHORUS IN THE LIVESTOCK INDUSTRY Union So Africa Dept Agr. Jour 8 460-504, illus 1924.

[4] Eckles, C. H, and Gullickson, T. W THE RELATION OF PHOSPHORUS DEFICIENCY TO THE UTILIZATION OF FEED Amer Soc Anim Prod. Proc 1927 16 21. 1928.

[5] Evans, R E THE INFLUENCE OF THE ADDITION OF CALCIUM CARBONATE TO A RATION LOW IN LIME ON THE APPETITE AND DIGESTIBILITY OF THE FOOD IN SWINE Jour Agr Sci [England] 19. [799]-801. 1929.

[6] Woodman, H E, and Evans, R. E NUTRITIVE VALUE OF PASTURE VI THE UTILIZATION BY SHEEP OF MINERAL-DEFICIENT HERBAGE. Jour Agr. Sci [England] 20 [587]-615. 1930.

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ceiving phosphorus supplement), a 3-year-old, weight 885 pounds, freshened September 14, 1929; (2) low-phosphorus cow, E-8, a 2-year-old, weight 845 pounds, freshened September 26, 1929; (3) low-phosphorus cow, E-9, a 2-year-old, weight 834 pounds, freshened October 7, 1929. All were good milkers, producing between 30 and 40 pounds of milk daily.

The animals were maintained as far as possible on a uniform plane of nutrition, which was 110 percent of the estimated digestible nutrients required as prescribed by the Morrison feeding standard. This intake was adjusted at the beginning of each month to meet changes in the milk production and live weight of the animals involved or as the circumstances of the experiment called for.

The typical ration furnished at the outset of the experiment was as follows: Prairie hay, 10 pounds; beet pulp (dry), 7 pounds; molasses, 3 pounds; blood flour, 1.6 pounds; grain mix (corn and oats), 3 pounds. This ration contained approximately 0.57 percent calcium and 0.12 percent phosphorus. With the phosphorus supplement (100 g monosodium phosphate) added, the phosphorus content was raised to 0.34 percent. While the calcium content of this ration was probably adequate, the phosphorus was present in subnormal amount.

Except during the colder months the cows were in dry lot most of the time when the weather permitted, being turned into the experimental barn only for feeding and milking.

Since the digestion and balance trial was to be run on animals in the phosphorus-deficient condition, the major concern was to induce aphosphorosis in the experimental animals through feeding the low-phosphorus ration. Although there was early evidence of phosphorus deficiency in both cows E-8 and E-9, the trial was not run until the latter part of June 1930. In the interval between the first marked symptoms of aphosphorosis and the period of the trials the low-phosphorus cows had received a phosphorus supplement on two occasions over extended periods, and it was not until the first part of June that they were judged to be again in a suitable condition of phosphorus deficit. Proof of this was furnished by the symptoms of depraved appetite, low efficiency of feed utilization, and also by the blood analysis. At the conclusion of the trial the inorganic phosphorus content of the blood of the experimental animals was as follows: Cow E-7 (control), 8.72 mg per 100 cc of whole blood; cow E-8 (low P.), 1.34 mg; cow E-9 (low P.), 1.2 mg.

Since the low-phosphorus cows would not consume the entire ration at all times because of loss of appetite, it was decided that during the 10-day preliminary period and the actual trial all 3 cows should be limited to an amount of feed which previous experience had indicated that they would completely consume. In this way the troublesome problem of refused feed was avoided. The amounts fed were as follows: Prairie hay, 4 pounds; beet pulp (dry), 4 pounds; molasses, 2 pounds; blood flour, 1.2 pounds; grain mix (corn and oats), 7 pounds. A sufficient supply of the several feeds was set aside at the outset of the digestion trial under such conditions that no significant change in moisture content would occur during the 10-day feeding period.

Complete collection of the excreta and milk was made. Toluene was used as a preservative in the collection of feces and urine. At the end of each 24-hour collection period an aliquot of the feces was taken and placed immediately in a drying oven. Aliquot samples of urine and milk were taken daily, preserved with toluene and formaldehyde, respectively, and kept in an ice box. The necessary chemical analyses for the calculation of digestibility of dry matter, crude protein, crude fiber, and the balance of calcium and phosphorus were made.[7]

PRESENTATION AND DISCUSSION OF DATA

The amounts of the different nutrients consumed, voided, and apparently digested are shown in table 1.

TABLE 1.—*Amounts of the different nutrients consumed, voided, and apparently digested by each cow during the trial*

Feed or cow no.	Dry matter	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
NUTRIENTS CONSUMED (GRAMS)					
Prairie hay	16,596	772	5,599	8,423	548
Beet pulp	16,725	1,476	3,345	10,771	105
Molasses	6,798	341		5,644	36
Blood flour	4,952	4,590	91	7	53
Corn and oats	28,716	3,397	950	22,564	1,330
Total	73,787	10,576	9,985	47,409	1,982
NUTRIENTS VOIDED IN FECES (GRAMS)					
E-7 (control)	22,440	5,015	4,066	9,774	549
E-8 (low P)	20,660	4,884	3,772	9,245	465
E-9 (low P)	23,320	5,087	4,533	10,469	554
NUTRIENTS APPARENTLY DIGESTED (GRAMS)					
E-7 (control)	51,348	5,561	5,919	37,634	1,433
E-8 (low P)	53,128	5,692	6,263	38,163	1,517
E-9 (low P)	50,468	5,489	5,452	36,940	1,429
COEFFICIENTS OF DIGESTIBILITY (PERCENT)					
E-7 (control)	69.6	52.6	59.3	79.4	72.3
E-8 (low P)	72.0	53.8	62.7	80.5	76.5
E-9 (low P)	68.4	51.9	54.6	77.9	72.1
Average for low-P cows	70.2	52.8	58.6	79.2	74.3

The digestion coefficients indicate that the ration employed in the trial was of average digestibility. The observed differences between the low-phosphorus animals and the control are not sufficiently marked or consistent to be regarded as significant. This reasonably close agreement between the coefficients of digestibility for the three animals supports the conclusion that phosphorus deficiency does not lower the animal's ability to digest its feed even when it is in an advanced stage of the disorder. From this it is evident that inefficient feed utilization in the phosphorus-deficient animal is not caused by any inability on the part of the animal to digest its feed properly. When the fact is considered that phosphorus is required in relatively small amounts in the processes of digestion, there is probably little reason why a shortage of this element in the ration should depress digestibility of the feed. Any deficiency in the ration is made up, undoubtedly, from the available reserves of the animal.

The data relating to the calcium and phosphorus balances of the experimental cows are given in table 2.

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[7] The analyses were made under the direction of Prof. W. L. Latshaw, of the chemistry department.

TABLE 2.—*Average daily calcium and phosphorus balances of experimental animals during digestion period*

CALCIUM

Cow no.	Feces	Urine	Milk	Outgo	Intake	Balance
E-7 (control)	26.9	0.4	7.8	35.1	41.5	+6.4
E-8 (low P)	27.1	5.0	9.7	41.8	41.5	−.3
E-9 (low P)	30.1	4.0	8.9	43.0	41.7	−1.3

PHOSPHORUS

E-7 (control)	25.5	1.3	7.6	34.4	39.6	+5.2
E-8 (low P)	10.3	.1	8.5	18.9	14.9	-4.0
E-9 (low P)	10.2	.1	8.1	18.4	14.9	-3.5

A consideration of the mineral-balance data brings out quite clearly the influence of the level of phosphorus intake on the calcium and phosphorus metabolism in the experimental animals. While cows E-8 and E-9 on a phosphorus intake of 14.9 g daily, as contrasted with 39.6 g for the control, showed negative daily balances of 4 g and 3.5 g, respectively, a positive balance of 5.2 g daily was recorded for E-7. It may be concluded, therefore, that the basal ration, while falling considerably short of phosphorus requirements, proved adequate and allowed for some storage when supplemented with 100 g of sodium phosphate daily.

It is interesting to note the uniformity of results for the different paths of outgo in the low-phosphorus cows. Apparently depletion of mineral reserves had reached approximately the same stage for each of these cows at the time of the trial. Attention is also directed to the greater urinary excretion of phosphorus in the case of cow E-7. The urine of the low-phosphorus cows was extremely low in this mineral, containing about one tenth that of the control. On the other hand, in the case of calcium just the reverse was true, namely, the urine of the control contained only approximately one tenth as much calcium as did the urine of the deficient animals.

The close association of calcium and phosphorus in metabolism is well illustrated in the data on the calcium and phosphorus balances. It will be observed that the negative phosphorus balances in cows E-8 and E-9 were accompanied by calcium balances of similar character, though not so marked in extent. On the other hand, the control cow, E-7, showed considerable storage of these minerals during the trial. It is evident that the heavy demand for phosphorus in the deficient animals must have resulted in a considerable break-down of skeletal material.

SUMMARY

The results of a study of the problem of lowered feed utilization in phosphorus-deficient cattle are reported.

Lactating dairy cows in advanced stages of aphosphorosis were found to digest their feed as efficiently as the normal control.

The lowered feed utilization by the phosphorus-deficient cattle did not result from inefficient digestion of the feed. Cows in this condition were found to be in negative balance for both calcium and phosphorus.

A STUDY OF THE IODINE CONTENT OF PENNSYLVANIA POTATOES[1]

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INTRODUCTION

This paper reports the iodine content of 135 samples of potatoes, of the 1931 crop, from 135 localities representing 62 of the 67 counties of the State of Pennsylvania. The possible relations of such iodine determinations to the soil types, fertilizer applications, variety and size of potatoes, and incidence of goiter on the farms on which the potatoes were grown and among the students of the Pennsylvania State College, are also pointed out.

The potato was chosen as the subject of this study because it probably forms a larger part of the diet of the people of the State than does any other single food except bread, because it is grown in all sections of the State, and because it is largely consumed in the locality in which it is produced.

The findings in such a study have greater significance for rural than for urban dwellers, as the former are much more likely than the latter to use locally produced potatoes as well as other foods, and the iodine content of potatoes is probably indicative—to some extent—of the iodine content of other foods and feeding stuffs grown in these localities.

The fact that deficient intake of iodine is the predominant cause of simple nutritional goiter has been thoroughly established through extensive surveys and experiments; but inasmuch as acute or chronic focal infection and generalized infection, such as influenza, may play a prominent secondary, or a relatively unimportant primary role of activation, in the production of thyroid disease (*3*; *7*; *8*; *17*, *p. 135*; *23*; *24*),[3] it is not to be expected that the iodine content of the diet will be found, under all conditions, in significant correlation with the incidence of goiter; and much less is it to be expected that the iodine content of a single food will be found to be so correlated.

The 135 iodine determinations reported, therefore, are presented mainly as indicating the value of potatoes from Pennsylvania as a source of iodine. There is some (though comparatively little) evidence of iodine deficiency in foods for human beings and livestock in that State.

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[1] Received for publication Sept 27, 1933; issued March, 1934. Technical Paper 604, Agricultural Experiment Station of Pennsylvania

[2] The author is greatly indebted to the county agricultural agents, and to the farmers who supplied the potatoes The author is also indebted to Dr. W. B. Mack and E P. Brasher for assistance in designing the apparatus; to O. J. Kahlenberg for cooperation in the iodine determinations; to Profs. F. G. Merkle and F. J. Holben for advice concerning soil types, and to Drs. J. P. Ritenour and C. D. Dietterich for the data on the medical examinations of students

[3] Reference is made by number (italic) to Literature Cited, p. 181

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METHOD OF SECURING AND PREPARING SAMPLES

In April 1931 each county agricultural agent in Pennsylvania was asked to name three farmers who would cooperate in furnishing potatoes for iodine analysis. The farms from which these potatoes were to come were to be selected as representative of the predominating types of soil and of farming conditions in each county.

As soon as replies were received from the county agents, a request for 20 average-sized potatoes was sent to each of the farmers named, with a questionnaire calling for information as to the exact location of the farm; the variety of potatoes; the amounts and kinds of commercial fertilizer used to grow the crop; and the incidence of goiter in the farmer's family and among his livestock.

In response to these requests 135 farmers sent potatoes. These samples were weighed in the original condition, and were then thoroughly washed, after which they were sliced and dried at 70° C. The samples were then allowed to come to equilibrium with the air with respect to moisture content, were ground, and enclosed in screw-cap fruit jars. Iodine and hygroscopic moisture were determined in samples weighed in the air-dry condition.

METHOD OF DETERMINATION OF IODINE

After a consideration of McClendon's method, as used by himself and Remington (*14*, *15*, *22*), the open-dish ashing method used by Remington, Culp, and Von Kolnitz (*21*), and the closed-combustion method of Karns (*11*, *12*), the last-mentioned was considered the most accurate, as determined by the recovery of added iodine. It was therefore adopted, in principle, but was modified to obviate the use of solid carbon dioxide and to lessen the cost and the time required in the operation.

The apparatus finally evolved is illustrated in figure 1. The only point of similarity between this apparatus and that of Karns is the combustion flask.[4] The flask used in these determinations was a Pyrex side-neck distilling flask of 2,000 ml capacity, with the side neck cut off to a length of about 6 cm. A Pyrex tube, bent at a right angle, was fused to the middle of the body of the flask, on the side opposite the side neck. This tube had an inside diameter of 8 mm and terminated at a point even with the mouth of the flask. A straight tube of Pyrex glass, of a diameter only slightly smaller than the inside diameter of the neck of the flask, and of such length as to extend from a point 6 cm beyond the end of the neck of the flask to a point 6 cm inside the body of the flask, was placed as indicated, and attached to the neck of the flask with thin-walled rubber tubing. A rubber stopper was fitted into the outer end of the straight tube mentioned; and through this stopper were passed a bent tube of small diameter for the introduction of carbon dioxide and a plunger for progressively raising the sample into the body of the flask as the combustion proceeds.

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[4] Mack and Brasher[5] evolved a flask similar to the one described, but with the oxygen inlet as well as the gas outlet fused into the bulb.

[5] Brasher, E. P. Thesis, Pennsylvania State College. 1932.

During the use of the combustion flask the side neck was connected with a cylinder of oxygen; the tube passing through the rubber stopper was connected with a cylinder of carbon dioxide; and the right-angle tube, attached to the body of the flask, was connected with the manometer and absorption train—as shown in figure 1. The absorption vessels were adaptations of the suggestion of Baumann and Metzger (*1*). These vessels consisted of a Witt filtering apparatus, within the ground-glass joint of which were placed three thicknesses of filter paper (or paper toweling) sufficiently porous to allow the passage of the gases, but serving effectively to condense and to retain the iodine. The two parts of the vessel were held together with clamps. With the apparatus described, 1 worker, using a duplicate set-up, can make 10 combustions a day without difficulty.

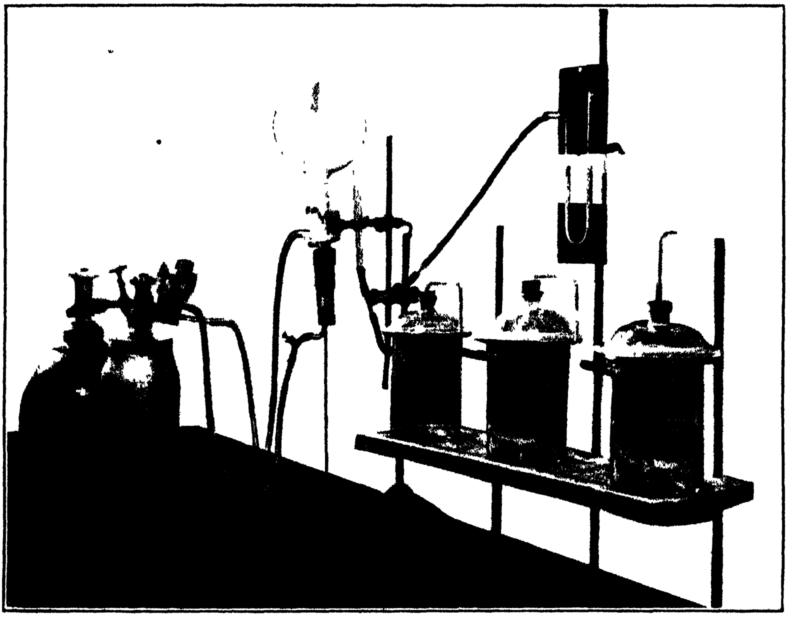

FIGURE 1.—Modified Karns apparatus for the determination of iodine.

In operation, the combustion flask, oxygen cylinder, and absorption train were connected as shown in the illustration, and a slow stream of oxygen was started into the flask. With the straight Pyrex tube outside the combustion flask, the sample, in a paper tube plugged at both ends with cotton, was introduced into the tube, and raised by means of the plunger until about 1 cm of the length of the paper tube protruded above the end of the Pyrex tube. The cotton at the end of the paper tube was then ignited; the Pyrex tube, carrying the paper tube of sample was quickly inserted in the flask; the rubber tubing which connected the combustion flask and Pyrex tube was then drawn into place, and the combustion was under way. From time to time, as the combustion proceeded, the sample was elevated by means of the plunger, to expose fresh material for combustion.

If the flame went out, as it sometimes did during the course of a combustion if the supply of oxygen was insufficient, the oxygen was turned off at once, and the carbon dioxide turned on, to extinguish the glowing fire in the sample. This obviated the danger of explosion.

After the last trace of combustible material had been burned, the apparatus was swept out with carbon dioxide. After several minutes of such sweeping, the apparatus was disconnected; the absorption train was washed out with hot water; and the filter paper was cut into strips and placed with the wash water in a beaker. The combustion flask was also washed out with hot water, and the washings, including the ash from the sample, were placed in a separate beaker. About 5 g of solid NaOH was added to the filter paper and washings, and the whole brought to boiling. The boiling mixture was filtered through paper in a Büchner funnel, and the paper thoroughly washed with hot water.

The washings thus obtained were combined with those from the combustion flask, and evaporated almost to dryness. The residue was then washed into an evaporating dish (nickel is preferable) and evaporated to dryness. To the residue was added approximately 5 g of NaOH in pellet form, and the whole was heated over a gas burner. Gentle heat was applied at first, but the temperature was gradually raised until fusion of the NaOH occurred, and the destruction of the organic matter was accomplished.

The melt in the dish was allowed to cool; distilled water was added, and the dish placed on the steam bath. When the contained solids had disintegrated to a pasty consistency the whole was allowed to cool and was extracted with 95-percent ethyl alcohol, previously purified by treatment with $AgNO_3$ and KOH, and distillation over KOH.

The extract (volume about 40 ml) was evaporated, and if the residue contained any organic matter this was fused again with an excess of NaOH, and extracted as before. The material thus extracted was washed into a 125-ml Erlenmeyer flask, and neutralized with dilute H_2SO_4 to the end point of methyl orange (aqueous solution). One small drop of bromine, purified by the method of Karns (*13*), was then added, together with a glass bead, and the mixture shaken until a distinct orange color developed. The bromine was then boiled off, and the solution cooled. A small amount of 20-percent solution of KI and 5 drops of starch solution were then added, and the solution titrated with N/1,000 sodium thiosulfate solution, prepared according to the method of Mayr and Kerschbaum (*16*).

DISCUSSION OF METHOD OF ANALYSIS

It is noteworthy that at no point subsequent to the combustion, which took place in a system designed to catch the liberated iodine, were the products containing the iodine exposed to a temperature much higher than the fusion point of NaOH (318° C.). Exposure to this temperature never occurred except in the presence of a considerable excess of NaOH, a condition which does not permit the volatilization of iodine compounds.

The efficiency of the filter papers (or paper toweling) in collecting the iodine from the gas stream was attested by recovery tests, the results of which are given in table 1.

TABLE 1.—*Recovery of iodine added to potato samples, by the modified Karns method*

Weight of sample (grams)	Iodine present in sample	Iodine added as KI	Iodine recovered	
	Gammas	*Gammas*	*Gammas*	*Percent*
53.3	6.9	50	49.6	99.2
52.1	6.8	60	57.5	95.8
50.9	6.2	100	94.2	94.2
49.4	6.2	100	98.2	98.2
49.0	6.2	100	87.8	87.8
56.1[1]	45	75.45	69.3	91.3
56.6[1]	45	75.45	72.9	96.0
Average				94.6

[1] These determinations were made after the completion of those on potatoes, the others were made before the determinations on potatoes had been made

While the recoveries here recorded are not perfect, they show that only approximately 5 percent of the added iodine was lost. All results reported are the mean of two closely agreeing determinations.

DISCUSSION OF RESULTS

The quantities of iodine found in the potatoes are shown in table 2. For convenience in comparison with other work these results will be discussed on the basis of air-dry material. The maximum quantity of iodine found was 197 parts per U.S. billion, and the minimum was 9 p.p.b. The mean iodine content of all samples, on this basis, was 70.7 p.p.b. On the basis of moisture-free material the maximum iodine found was 216, the minimum was 10, and the mean, 77.8 p.p.b. These values are of about the same magnitude as those reported by Orr (*18*), who found a minimum of 22, and a maximum of 251 p.p.b., for potatoes grown in different parts of England and Scotland. They are considerably lower, however, than the figures reported by Remington, Culp, and Von Kolnitz (*21*), who found that potatoes from South Carolina varied in iodine content between 87 and 554 p.p.b., with an average of 217 p.p.b. in 72 samples. Values for iodine in potatoes compiled by Orr and Leitch (*20*) include analyses by Von Fellenberg (*4*) and by Hercus and Roberts (*10*). These results, together with some analyses made by Orr and Leitch, are on the fresh basis, but assuming 25 percent of dry matter, the values reported range from 16 to 140 p.p.b. Bleyer (*2*), in some analyses reported by Orr and Leitch, found between 80 and 136 p.p.b., and Brasher[6] found between 150 and 240 p.p.b.

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[6] BRASHER, E. P. See footnote 5.

TABLE 2.—*Iodine content of potato samples from various Pennsylvania counties*

Sample no.	County of origin	Iodine		Sample no.	County of origin	Iodine	
		Air-dry basis	Moisture-free basis			Air-dry basis	Moisture-free basis
		P.p.b.	*P.p.b.*			*P.p.b.*	*P.p.b.*
1	Centre	46	50	69	Somerset	48	54
2	Crawford	61	67	70	Clinton	9	10
3	Elk	46	51	71	Pike	90	103
4	Perry	101	113	72	Lehigh	66	74
5	Cameron	97	106	73	do	101	113
6	Mercer	116	129	74	Snyder	59	64
7	York	139	149	75	Beaver	111	125
8	Juniata	138	158	76	Lycoming	66	72
9	Bradford	99	110	77	Forest	22	24
10	Luzerne	109	123	78	Cambria	119	132
11	Lancaster	122	133	79	Butler	32	35
12	Snyder	56	62	80	Forest	91	101
13	Indiana	134	148	81	Erie	77	8[illegible]
14	Somerset	83	89	82	Venango	71	80
15	Monroe	103	113	83	Union	91	101
16	Indiana	197	216	84	Butler	95	105
17	Cumberland	61	67	85	Clearfield	40	44
18	Lackawanna	49	55	86	Potter	50	55
19	Luzerne	148	163	87	Carbon	10	11
20	Warren	99	109	88	McKean	43	47
21	Schuylkill	106	118	89	Luzerne	67	76
22	Bedford	86	94	90	McKean	14	15
23	Wayne	60	65	91	Centre	57	62
24	Mifflin	116	125	92	Crawford	20	22
25	Wayne	48	52	93	Sullivan	86	94
26	Dauphin	---	-	94	Northampton	99	106
27	Cameron	32	35	95	Armstrong	56	60
28	Clearfield	35	38	96	Bradford	22	25
29	Allegheney	20	22	97	Clearfield	66	73
30	Fayette	66	75	98	Bedford	103	111
31	Bucks	73	80	99	Huntingdon	47	53
32	Union	40	44	100	Adams	58	63
33	Northumberland	66	73	101	Chester	67	73
34	Huntingdon	155	167	102	Fayette	26	29
35	Venango	169	192	103	Beaver	19	21
36	Northampton	63	68	104	Bradford	61	66
37	Perry	73	79	105	Crawford	101	100
38	Dauphin	85	94	106	Susquehanna	70	76
39	Northumberland	58	65	107	Clarion	54	59
40	Mifflin	26	29	108	Schuylkill	61	65
41	Philadelphia	162	178	109	Mercer	82	88
42	Delaware	74	80	110	Elk	123	134
43	York	33	37	111	Wyoming	53	58
44	Lebanon	83	91	112	Berks	39	42
45	Delaware	122	135	113	Beaver	44	48
46	Philadelphia	77	86	114	Adams	58	63
47	Lancaster	45	50	115	Northumberland	55	61
48	Lycoming	17	18	116	Pike	127	136
49	Warren	92	100	117	Blair	76	82
50	Elk	27	30	118	Northampton	26	29
51	Juniata	61	68	119	Cambria	29	32
52	Erie	140	151	120	Sullivan	53	60
53	Mifflin	11	12	121	Wayne	14	16
54	Blair	148	161	122	Cameron	36	40
55	Lawrence	92	102	123	Clinton	39	42
56	Potter	45	50	124	Tioga	37	42
57	Bucks	39	42	125	Greene	18	20
58	Venango	75	81	126	Monroe	57	64
59	Erie	167	185	127	Chester	38	42
60	Berks	60	65	128	Columbia	86	96
61	Lebanon	47	53	129	Armstrong	69	74
62	Perry	43	46	130	Franklin	19	21
63	Lycoming	85	92	131	Wyoming	24	27
64	Lackawanna	100	107	132	Franklin	112	126
65	McKean	71	77	133	Allegheney	155	166
66	Bucks	126	138	134	do	57	61
67	Westmoreland	60	66	135	Blair	21	23
68	Clinton	26	29	136	Franklin	48	52

RELATION OF IODINE CONTENT OF POTATOES TO GEOGRAPHICAL DISTRIBUTION

For the consideration of the iodine content of the potatoes as related to the locality of origin the iodine values were grouped and averaged to represent six arbitrary divisions of the State, as indicated in figure 2. Potatoes from the north-central part contained significantly less iodine than did potatoes from the other sections. The mean difference between the iodine content of potatoes grown in the north-central and those grown in the southeastern division was 31±8 p.p.b., a figure which may be considered significant, since the odds against such a difference occurring by chance alone are 100 to 1. The differences between the iodine content of the potatoes from the north-central section and those grown in the other four divisions of the State are not so significant, in the light of such statistical analysis, even though as in the case of the northwestern section, the numerical difference is greater.

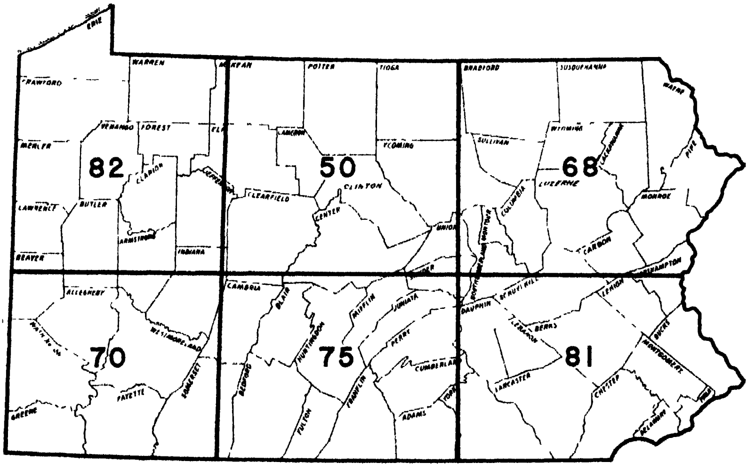

FIGURE 2.—Relation of iodine content in parts per billion of samples of potatoes to geographical origin.

RELATION OF IODINE CONTENT OF POTATOES TO SOIL TYPE

The potato samples were divided into groups according to the soil types on which they were grown. There are two predominant soil types in Pennsylvania, a nonglaciated, residual soil, derived from sandstones and shales, which comprises about 62 percent of the area of the State and extends over the central and southwestern portions; and glaciated soils of similar origin found in the northwestern and northeastern sections of the State, and covering about 24 percent of the total area.

A third type of soil is derived from calcareous rocks, and lies mainly in the long valleys of the south-central and southeastern sections; a fourth type of soil, derived from igneous rocks, is found mainly in the southeastern section of the State; and a fifth type is of marine origin, and lies in a narrow strip along the extreme southeastern border of the State.

The average iodine contents of the potatoes from these soil types are shown in table 3. The probable errors of the averages of groups 3, 4, and 5 are presented because of the small numbers of samples.

TABLE 3.—*Iodine content of potato samples grown on various soil types in Pennsylvania*

Soil number and type	Samples	Mean iodine content, air-dry basis
	Number	*P.p.b.*
1. Dekalb and Westmoreland	74	66±4
2. Volusia and associated groups	30	79±6
3. Hagerstown	18	63
4. Penn and Lansdale	6	74
5. Sassafras (marine)	2	142

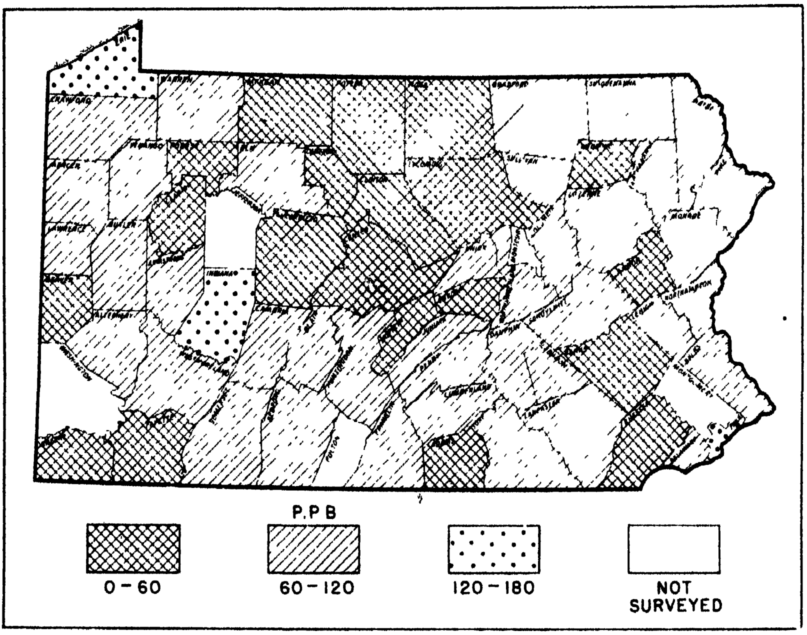

FIGURE 3.—Relation of iodine content of samples of potatoes to their counties of origin.

No significant differences were found between the average iodine contents of the potatoes from the different soil types except that the two samples grown on marine soil were unusually high in iodine.

Figures 3 and 4 represent the distribution of the iodine of the potato samples by counties as compared with a generalized soil map of the State. The potatoes from the north-central area of nonglaciated soil were comparatively low in iodine—as already noted—but as many of the apparently low-iodine counties were outside this area as were within it.

VARIETY OF POTATOES AND IODINE CONTENT

More than 82 percent of the potatoes studied were of some strain of the Russet variety, and about 10 percent were of the Rural New Yorker variety. The average iodine content of those potatoes belonging to the former variety was 72 p.p.b., while the average iodine content of the Rural New Yorker variety was 67.4 p.p.b. This difference is not significant.

RELATION OF SIZE OF POTATOES AND IODINE CONTENT

Since whole potatoes have been shown to contain more iodine than those which have been peeled, it was considered possible that some relation might exist between size (and indirectly the ratio of surface to mass) and the iodine content, but the statistical treatment of the data revealed no such relation.

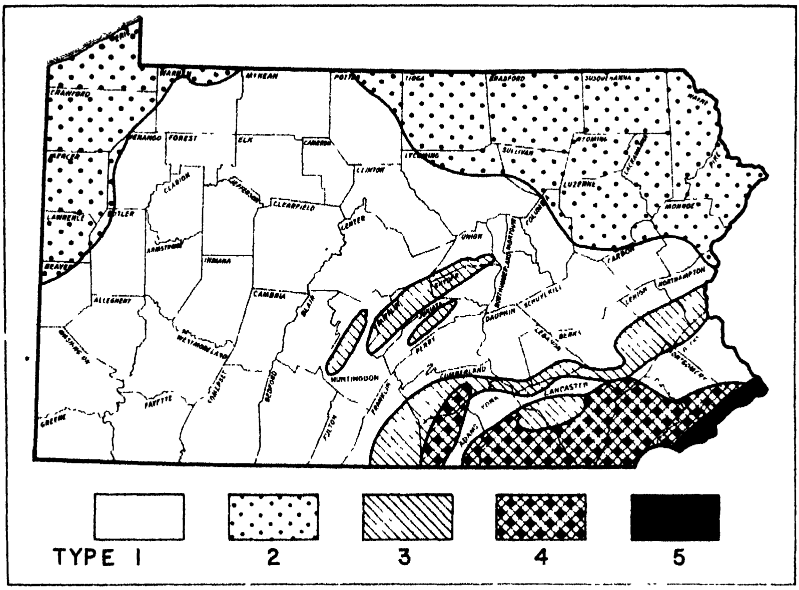

FIGURE 4. Generalized soil map of Pennsylvania.

RELATION OF IODINE CONTENT OF POTATOES TO FERTILIZER APPLICATIONS

Numerous workers, including Orr, Kelley, and Stuart (*19*), Von Fellenberg (*5*, *6*), Hercus and Roberts, (*10*), and Hercus, Benson, and Carter (*9*) have shown that iodine applied to the soil may be taken up by the plant, and that some fertilizer materials, notably Chilean sodium nitrate, contain appreciable quantities of this element. In this study, the samples of potatoes were grouped according to the amounts of nitrogen applied to the crop as commercial fertilizer, but this grouping brought to light no significant relation—as might have been expected in view of the fact that the source of the nitrogen of the fertilizers was not known.

RELATION OF IODINE OF POTATOES TO INCIDENCE OF GOITER

Twenty-four of the 135 farm families which raised the potatoes used in this study reported one or more cases of goiter. Five of these families had 2 cases, there being a total of 29 cases among approximately 675 individuals, or an incidence of 4.3 percent. Attention is called to the fact that these reports were made by the farmers themselves, and minor thyroid enlargements may have passed unnoticed.

Only 6 instances were reported of thyroid derangement among the livestock of the 135 farms.

The iodine content of the potatoes grown on farms on which the farm families had one or more cases of goiter was 87 p.p.b., while the potatoes from the other farms showed an iodine content of 76 p.p.b., both figures being on a moisture-free basis.

RELATION OF IODINE OF POTATOES TO INCIDENCE OF GOITER AMONG FRESHMEN AT THE PENNSYLVANIA STATE COLLEGE

The records of the physical examinations given each of the past five incoming classes at the Pennsylvania State College were examined to determine the incidence of goiter among the 6,354 individuals composing this group. In other studies of this group it has been determined that the rural-urban distribution among these students agrees very closely with such distribution of the population of the State as a whole, as determined by the census figures. Of the 6,354 individuals examined, 5,405 were males and 950 were females.

The frequency of goiter among the student population included in this survey was 2.9 percent. Several other surveys made in past years by various individuals connected with the Pennsylvania Department of Health indicate a state-wide incidence of goiter of between 2 and 3 percent.[7]

In consideration of the slight incidence of goiter in the State, and the uncertain significance of the iodine content of potatoes as indicative of the iodine content of the diet as a whole, no significant correlation was to be expected from this comparison, and none was found.

SUMMARY

One hundred and thirty-five samples of potatoes, grown in as many localities in Pennsylvania, were analyzed for iodine by a modification of the method of Karns. The minimum iodine content was found to be 10 p.p.b., the maximum 216 p.p.b., and the mean 77.8 p.p.b., expressed on a moisture-free basis.

The mean iodine content of the potatoes grown in the southeastern section of the State was found to be significantly higher than that of the potatoes grown in the north-central section, but no other sectional differences in iodine content were significant.

The analyses of potatoes grown on five different soil types in the State showed that those grown on glaciated soils contained slightly more iodine than did those grown on similar nonglaciated types. Two samples of potatoes grown on marine soil showed a much higher iodine content. The iodine content of the potatoes analyzed was apparently not affected by the fertilization.

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[7] WOOD, H. B. Personal communication.

No significant correlation was found between the iodine content of the potatoes and the size of the individual potatoes, nor between the iodine content and variety.

Medical examination of 6,354 students at the Pennsylvania State College during the past 5 years showed a goiter incidence of 2.9 percent. No significant correlation was found between the iodine content of the potatoes from the different parts of the State and the incidence of goiter in the same localities, as indicated by the examination of these students.

A similar study of the relation of the iodine content of the potatoes and the incidence of goiter among the 135 farm families showed no significant relation between the two. The goiter incidence in the farm families was 4.3 percent. The potatoes from farms upon which there were one or more cases of goiter showed a considerably higher iodine content than those from farms on which no goiter was reported.

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COMPARATIVE VALUE OF SOME COMMERCIAL PROTEIN SUPPLEMENTS IN THE RATIONS OF GROWING CHICKS[1]

By O. Johnson, *assistant chemist*, and D. Brazie, *assistant poultry husbandman, Washington Agricultural Experiment Station*

INTRODUCTION

Among the more important sources of protein concentrates available to the commercial poultrymen in the State of Washington are milk products, trimmings from meat-packing plants, and byproducts from fish canneries. Wide variations have been observed in the results obtained by the use of these different concentrates. The experiment here recorded was conducted to determine the feeding value of these different protein concentrates and blends of these concentrates when used in chick rations recommended by the State College.[2]

EXPERIMENTAL METHODS AND MATERIALS

A group of Single Comb White Leghorn chicks was obtained from commercial hatchery stock. From this group, 9 lots of 20 chicks each were so selected that at the beginning of the trials the chicks in all lots possessed uniform physical characteristics. The lots of chicks were brooded for 8 weeks under the conditions described by St. John, Carver, Helphrey, Miller, and Cassel.[3] The feed consumption and individual chick weights at biweekly periods were recorded and observations were made on the physical condition and mortality of the chicks. The excreta were collected daily and sampled according to the method described by St. John and Johnson.[4]

The all-mash basal ration was made up as follows: 40 pounds ground yellow corn; 20 pounds ground wheat; 10 pounds ground oats; 10 pounds white-wheat bran; 4 pounds dehydrated alfalfa; 1.5 pounds steamed bone meal; 1.5 pounds oyster-shell flour; 0.75 pound salt; and 0.25 pound cod-liver oil. The protein concentrates were adjusted to a 34 percent protein level with cornstarch, so that 12 pounds of the diluted concentrate with 88 pounds of the basal ration gave a protein content of approximately 14 percent for the completed ration.

Previous investigations at this station by Carver, St. John, Aspinall, and Flor[5] have shown that 14 percent of protein is close

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[1] Received for publication June 7, 1933; issued March, 1934. Published as Scientific Paper no. 263, College of Agriculture and Experiment Station, State College of Washington.

[2] This work was done cooperatively by the Divisions of Poultry Husbandry and Chemistry.

[3] St John, J L, Carver, J S, Helphrey, J P., Miller, W., and Cassel, L W. THE EFFECT ON GROWTH OF VARIOUS PROTEIN LEVELS OF DRY SKIM MILK IN A CHICK MASH. Poultry Sci. 9: 320 333, illus. 1930.

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to the lower level of protein consistent with optimum growth, and therefore variations in the value of the various concentrates should bring out differences which would not be apparent if the percentage of protein were closer to the excessive level. Table 1 gives the data on the rations used for each lot of chicks.

Table 1.—*Analyses (percent) of the complete ration composed of 88 pounds of basal ration and 12 pounds of the mixture of concentrate and cornstarch*

Lot no.	Protein concentrate	Protein in concentrate	Protein in total ration	Ash in total ration
70	Argentine meat scrap	41.60	14.22	9.10
71	Vico meat scrap	61.70	14.40	6.38
72	Alaska herring meal	73.00	14.30	5.47
73	Fish shreds	59.00	14.20	6.65
74	Skim-milk powder	36.50	14.57	5.93
75	Blend 4 percent each Argentine meat scrap, herring meal, skim-milk powder		14.10	6.76
76	Blend 4 percent each Argentine meat scrap, herring meal, skim-milk powder; minerals changed to 1 percent oyster-shell flour, 2 percent cornstarch		14.10	5.24
77	Blend. 4 percent each Argentine meat scrap, herring meal, skim-milk powder, minerals changed to 2 percent oyster-shell flour, 1 percent cornstarch		13.65	5.94
78	Blend 4 percent each Vico meat scrap, fish shreds, skim-milk powder		14.35	6.23

RESULTS

In considering the efficiency of the various protein concentrates used, the most important criterion from the commercial poultryman's point of view is the actual growth response of the chicks. The growth curves for the 8-week periods are shown in figure 1. To facilitate comparison, these curves are presented in the order of descending value.

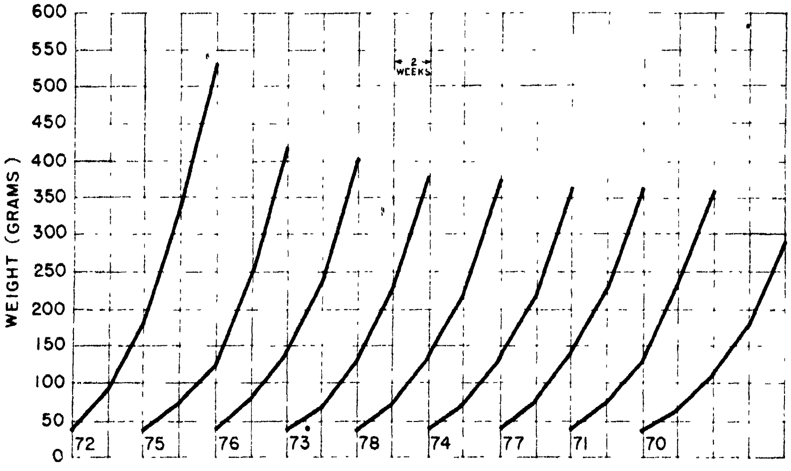

Figure 1.—Growth of chicks when fed various protein concentrates (indicated by numbers) for a period of 8 weeks.

The growth of the chicks in lot 72 is strikingly better (525) than that in any of the other lots, while the growth in lot 70 is decidedly poorer (289). The other lots have a medium value between these two, ranging from 363 to 414 g. Miller and Bearse[6] reported from the Western Washington Experiment Station, a similar trial in which a ration containing 17 percent of protein was used and 14 hours of light, as compared with 14 percent of protein and 12 hours of light in this test. They obtained better growth for all lots. The lower level of protein employed in the present experiment is perhaps responsible for the smaller growth obtained, but this low level of protein would also accentuate any differences in the comparative feeding value of the different protein supplements used. In the general appearance of the birds there were no marked differences between lots other than those to be expected in view of the differences in growth. From the growth response of the birds it would seem that herring meal, the protein supplement used in the ration fed lot 72, was a much better supplement than the other concentrates, and superior particularly to the Argentine meat scrap used in the ration fed lot 70.

The biological values for the protein supplements fed the different lots of chicks were determined by the method described by St. John, Johnson, Carver, and Moore.[7] These values are given in table 2.

Table 2.—*Biological values of protein supplements, based on average chick weights for the 2-week periods*

Period	Biological value of protein supplement fed to lot no.—								
	70	71	72	73	74	75	76	77	78
First 2 weeks	62.9	56.3	63.5	59.2	61.4	58.1	62.9	65.4	67.5
2 to 4 weeks	62.7	59.8	61.5	55.2	58.5	65.2	58.8	67.0	70.8
4 to 6 weeks	62.3	55.6	64.4	59.1	65.3	57.6	62.6	65.9	61.0
6 to 8 weeks	71.7	72.1	71.2	63.0	64.9	69.1	63.2	70.0	70.8
Average	64.9	61.0	65.1	59.1	62.5	62.5	61.9	67.1	67.5

There does not appear to be any significant relation between the actual percentage of protein retained by the chicks and their growth. When the gain in weight per gram of protein ingested is considered, however, there appear variations which are significant. The gains in weight per gram of protein ingested are given in table 3.

The chicks in lot 72 made the greatest gain in weight per gram of protein for the first 6 weeks. The biological values of lot 72 did not differ greatly from those of lot 70, which made the lowest gain per gram of protein and also showed the poorest growth response. The chicks in all lots had free access to an unlimited feed supply, since it was desired to study their reaction under conditions as nearly comparable to those in the field as would be consistent with controlled experimental procedure. The amount of feed consumed by the chicks varied greatly between lots and the variations in growth of the chicks in the different lots may be explained on this basis.

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TABLE 3.—*Gain in chick weight (grams) per gram of protein consumed, and average weight (grams) of feed consumed per chick, when various protein supplements were fed*

Period	Lot 70	Lot 71	Lot 72	Lot 73	Lot 74	Lot 75	Lot 76	Lot 77	Lot 78
GAIN IN WEIGHT									
First 2 weeks	2.02	2.43	2.66	2.34	2.34	2.48	2.63	2.32	[illegible]
2 to 4 weeks	1.75	1.82	2.34	2.12	1.96	2.28	2.03	1.86	1.84
4 to 6 weeks	1.86	2.11	2.48	2.20	1.97	2.09	2.26	1.72	1.82
6 to 8 weeks	1.31	1.64	1.78	1.74	1.75	1.76	1.97	1.73	1.79
Average	1.70	1.87	2.14	2.03	1.92	2.08	2.19	1.81	1.85
FEED CONSUMED PER CHICK									
First 2 weeks	103.3	109.9	134.7	102.8	107.5	102.3	111.8	119.8	107.0
2 to 4 weeks	178.4	211.4	265.8	193.6	201.4	215.4	210.4	245.2	211.2
4 to 6 weeks	283.2	330.1	421.9	319.1	296.8	361.7	307.3	363.6	343.2
6 to 8 weeks	552.1	565.5	780.0	602.7	598.6	663.6	596.2	608.4	612.6
Average	1,104.0	1,214.9	1,596.2	1,179.8	1,185.8	1,333.1	1,184.8	1,337.0	1,274.0

The birds in lot 72 consumed more feed and made greater gains per gram of protein ingested than those of any of the other groups. The amount of feed consumed by lot 70 while the lowest for all the groups, was not sufficiently low to account for the poor growth of this pen. In the case of lot 70, poor growth may have been due to the high ash content of the ration. Frasier and Annonen,[8] using the same kind of meat scrap that was employed in this study, found evidence of a close association between high-ash content and low chick weight. As the analyses in table 1 show, lot 70 is the only lot to which this conclusion can be applied. Factors other than protein that might have influenced the results of this study are now being investigated.

SUMMARY

A study was made to determine the value of various protein concentrates used as supplements to chick rations. The protein in the ration was maintained at approximately 14 percent for all lots of chicks.

The chicks fed Alaska herring meal made the best growth, those fed Argentine meat scrap made the poorest, while those fed the concentrates and blends of concentrates showed a growth about midway between the two.

The biological values were determined for each lot of chicks, but could not be associated with other data to show that the better growth in some lots was due to a better utilization of the protein in the ration.

While the lot showing the greatest gain in weight per gram of protein ingested made the best growth, this association did not hold for the other lots. The lot making the greatest gain per gram of protein also showed the greatest feed consumption.

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[8] FRASIER, F. W., and ANNONEN, W. BIOLOGICAL VALUES OF FISH MEALS AND MEAT MEALS. Unpublished thesis. State College of Washington. 1932.

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STUDIES ON ARMILLARIA MELLEA (VAHL) QUEL., INFECTION, PARASITISM, AND HOST RESISTANCE[1]

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INTRODUCTION

Armillaria mellea has long been recognized as an important plant parasite, mainly attacking forest and fruit trees and causing in them a serious root rot. It grows luxuriantly as a saprophyte on the dead roots and stumps of trees and, according to Kusano (*25*),[2] may also act in a mycorrhizal relationship.

A great deal of effort has been expended upon control measures in many parts of the world, but with little success. Much has been said about resistant rootstocks, but practically nothing is known regarding the nature of resistance to this fungus. The present work had as its object a study of the mode of entrance, the resistance of various rootstocks, and the nature of such resistance. It was believed that more was to be gained by examining a considerable number of different hosts than by confining the investigations to a more exhaustive study of one susceptible and one resistant host. The principal part of the study dealt with a histological and cytological investigation of the mode of infection of susceptible and resistant hosts. The term "infection" as here used means either the penetration of the fungus into the host without further development, or penetration with subsequent production of a diseased condition. Further work was undertaken on the nature of resistance.

Not much definite knowledge exists as to susceptible and nonsusceptible roots or tubers. In this study those rootstocks or plants which usually escape destruction in "*Armillaria* spots" (soil areas where the disease occurs) will be considered as resistant, and those usually destroyed as susceptible.

REVIEW OF LITERATURE

The method of invasion of tree roots by *Armillaria*, whether through wounds or through the healthy bark, received some attention from the early German workers. Hartig (*14*, *15*) in 1873 and 1874 was the first to establish the fact that *Armillaria mellea* (then called *Agaricus melleus*) was the cause of a serious root disease, which had been known for some time but which was believed to be due to an excess of resin. He further proved the connection between the rhizomorphs and the fruiting bodies. At that time he was unable to determine whether the fungus entered through a sound root of the attacked conifer or through a wound. He observed, however, that when trees were close together none escaped the disease. At a later date he (*16*) concluded that the rhizomorphs penetrate the sound roots of conifers and that wounds in the roots are not necessary to allow entrance; he gave no details of the fungal invasion. He was uncertain as to the entrance of the fungus into plum and cherry but considered it a parasite on them. In 1894, after experimenting with oaks in which he cut the top off and allowed the roots to sprout, Hartig (*17*) concluded that *Armillaria* is a wound parasite and is unable to enter uninjured roots, that if the tissue is in an active growing state the fungus does not invade it, and that only resting tissues are attacked. Later work (*18*), however, caused him to modify his view. When healthy oaks had a single root removed and left for 2 years before being examined, it was found that the cuts were covered with rhizomorphs, that the fungus had penetrated for a depth of 3 mm, and was there held in check with no further penetration into the wood or cortex. From this he concluded that the fungus was not a parasite on a healthy oak, not even a wound parasite.

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[1] Received for publication July 26, 1933; issued April 1934. Contribution from the Division of Plant Pathology, University of California, Berkeley, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 216.

Brefeld (*6*) developed methods for growing this fungus in pure culture and used these cultures to infect thick, freshly dug roots of the Scotch pine, on which he stated the fungus most often occurred parasitically. He dug out and brought the roots "uninjured and fresh" into direct contact with the tips of the rhizomorphs. Penetration occurred at once, and the fungus emerged at the cut end between the bark and wood after 5 to 7 days. The act of penetration was said to require 1 to 2 days, but no description or illustration of the exact process is given. It is stated that the rhizomorph as it creeps over the surface of the root forms lateral branches which penetrate directly into the root; or the tip itself may enter. Hiley (*21*) and Zeller (*46*) do not believe this method to be capable of testing the normal rhizomorphic invasion into healthy, uninjured roots. They believe it is impossible to remove roots from the soil without some undetectable surface injury and do not consider roots to be in a healthy, living condition when so treated. Evidence obtained in the examination of Austrian forests is presented by Cieslar (*10*) who concluded from his field observations that entrance by *Armillaria* into the roots of oak, ash, and elm is gained through wounds in the crown or the roots below it. The fungus sometimes found in irregularities on the surface of oak and elm roots was always walled off by a periderm layer before it penetrated to the cambium. He notes the importance of insect injuries as a means of gaining entrance. De Bary (*4*) in 1884 stated that the strands penetrate into the healthy living cortex of roots of healthy trees, especially the conifers, but he probably accepted Hartig's views.

It thus appears that the early German workers were fairly well agreed that the fungus can enter the sound living bark of conifers but can only enter the broadleaf trees through some wound in th bark. They give none of the details of actual penetration nor do they state how the fungus enters the sound cortex. Brefeld makes the statement that branches of the rhizomorph form on the lower side and penetrate directly but does not state how the entrance takes place.

Neger (*32*) in 1908 very briefly described the penetration of the rhizomorph into the roots of the silver fir. He found, in trees displaying a dying-back of the lower branches, a network of rhizomorphs surrounding the taproot and in some places entering the bark. At such points an appresorium or suction plate had formed, from which the penetrating rhizomorph branch grew. The latter first enters the outer dead portion of the bark and later pushes through the cork into the tissue below. New and deeper layers of cork then form, but Neger observed that these are often in turn penetrated by the rhizomorph.

In 1911 Kusano (*25*) investigated the relation of *Armillaria mellea* to a Japanese orchid, *Gastrodia elata*, to which the fungus acts in a mycorrhizal connection. He states that the rhizomorph grows on the surface of the tuber, fastens itself to it, and sends infection branches into the tissues of the tuber. In so doing the fungus first dissolves the outermost suberized cork cells and then by dissolution of the dividing walls attacks the underlying living cells forming a "lysigenic space for the advance of the growing infection strand." The single hyphae which composed the infecting rhizomorph then enter the surrounding cells as single mycelial threads and no longer maintain the rhizomorphic structure. The symbiosis is established. Kusano further observed that the fungus may at times attack the *Gastrodia* tuber parasitically and in so doing causes compression and a brownish discoloration of the cells surrounding the infecting strand. This is not apparent in the symbiotic relation. During the investigation Kusano also found the fungus attacking potato tubers parasitically. He concludes that the action on the potato is much the same in its general aspect as the parasitic action on the *Gastrodia* tuber. The work of Kusano applies to such parenchyma tissue as that of a tuber.

It cannot be assumed, without further proof, that entrance into the hard tissue of a root takes place in exactly the same manner. Horne (*22*) has studied the general aspects of the *Armillaria* problem, probably more thoroughly than any other American worker, but he has dealt with the subject mainly from a practical point of view and not from the standpoint of detailed microscopical study. As to penetration he suggests that—

> when the tip of the rhizomorph comes to a healthy root the very small microscopical threads of which it is composed, seem to loosen like the cut end of a rope and the individual threads penetrate into the bark and start a new infection.

It would be assumed from this that the author believes penetration is through the sound healthy bark. Nechleba (*31*), observing the behavior of *A. mellea* in forest trees, considers wounds or a decrease in the vigor of the host essential to entrance of the fungus. Barss (*3*) believes that rhizomorphs may penetrate into healthy bark but more often gain entrance through injured roots or crowns. Hiley (*1, p. 159*) studied the *Armillaria* root rot of the larch under forest conditions in England. He makes some rather sweeping statements regarding the penetration of fungi into tree roots.

> Though the early pathologists seem commonly to have accepted the theory that fungi pierce the sound bark of trees, no authenticated instance of this has ever been recorded, and the trend of recent opinion has been more and more in the direction of admitting the possibility of infection only by wounds or by outflanking the bark protection.

Hiley further states: "The mode of infection employed by *Armillaria mellea* has never been critically examined * * *" and considers the problem a difficult one to elucidate. He maintains that with rhizomorphic infection it is "* * * still a question whether entrance is effected (i) through healthy, uninjured bark, (ii) through wounds, or (iii) through dead roots", and that his observations support the view that rhizomorphs can enter only through damaged or dead roots. Samofal (*36*) concluded after 20 years' observations in the pine forests in the Provinces of Chernigof and Kief in Ukraine that *A. mellea* seems to be a parasite and saprophyte on conifers and only a saprophyte on broadleaf trees, gaining entrance to the latter when the roots have been injured.

Zeller (*46*) in 1926 concluded that in rhizomorphic infection, Horne's idea of penetration in which the single threads of the rhizomorph strand enter individually "comes the nearest to describing actual infection." He observed that healthy roots during their growth sometimes come into very close contact with diseased roots and that the healthy roots become diseased at the point of contact. Without following the actual process he postulates that toxic substances produced by the growth of the fungus in the diseased root, upon coming in contact with the healthy bark, enter through the lenticels, causing a shallow disorganized spot in the tissue which blisters and later flakes with the development of cork under it. The process is repeated until several layers of flakes form and the fungus finally passes into the cambium. He thinks this action resembles a type of "burning" which may occur when organic debris is brought into close contact with young bark. He believes that further evidence of the toxic substances produced by this decay is seen in the effect on the top of the tree.

Another theory on penetration advanced by Zeller deals with the entrance of the vegetative mycelium of *Armillaria* at the point of emergence of lateral roots, through the rupture in the bark parenchyma made by the root in forcing through. From the theories presented by this author it is evident that he considers a wound or other disturbance necessary before *Armillaria* can enter. The method of entrance by contact of diseased and healthy roots is alluded to in the writings of other authors, among whom are Lawrence (*26*), Barrett (*2*), Birmingham (*5*), Hendrickson (*20*), and Samofal (*36*); and the phenomenon has undoubtedly been observed by many others who make no mention of it. Old roots or root pieces may live for many years in the soil and remain as a source of infection by this method.

While the experimental work reported on later in this paper was in progress an article by Day (*12*) appeared, which did not come to the attention of the author until this work was completed. In this article Day describes and illustrates the mode of entrance employed by the rhizomorph in the infection of conifers and discusses their susceptibility to attack. He examined and studied naturally infected roots taken from the forest and in so doing recognized the possibility that "it would perhaps have been better had it been collected from such trees specially inoculated with, or exposed to, attack by a pure culture of the fungus." Day concluded that the attack of *Armillaria mellea* is solely by rhizomorphs. Attachment of this organ to the host is quite apart from penetration and occurs when the rhizomorph grows into the dead, outer cork cells. The fungus appears to exert a toxic influence upon the host tissue under the rhizomorph as a preliminary to penetration. The rhizomorphs penetrate the cork by dissolution instead of by mechanical rupture. A toxic influence precedes the advance of the rhizomorph. Secondary cork layers form to prevent entry of the parasite. This process continues in some instances and invagination results. The evidence indicates that *A. mellea* is able to penetrate an uninjured and apparently healthy host. Observations in the forest led this author to the opinion that variations in susceptibility to attack among species of conifers do not coincide with their susceptibility to death after attack and is possibly accounted for by external environmental factors. Day has undoubtedly contributed more than any other worker to the solution of the problem of the method by which *A. mellea* enters its hosts. His work is, however, concerned only with coniferous roots.

Rayner (*35*) using a pure culture of *Armillaria mellea* failed to obtain satisfactory infection in inoculated seedling Corsican pine and Douglas fir grown aseptically in sand. Under the conditions of the experiment the fungus did not organize rhizomorphs, and it is stated only small cankers, subsequently exfoliated, were produced by the action of single mycelial threads. Such cankers on roots were practically confined to the neighborhood of emerging laterals.

From this review it is seen that there still exists some dispute concerning the details of infection, at least in woody plants and trees; that no critical microscopical studies have been reported regarding the mode of entrance into tree roots under controlled conditions; that no systematic attempt to investigate and compare penetration into the so-called resistant and nonresistant rootstocks has been reported; and that little or nothing is known regarding the nature of host resistance.

METHODS

The culture of *Armillaria mellea* used throughout this study was made from a root of an old prune tree (*Prunus domestica*) killed by the fungus. A culture was thus obtained from a strain known to be parasitic, which might not be the case if a single-spore culture had been used. The culture has been maintained on prune agar since isolation. Material used for soil inoculation was cultured on prunings from fruit trees autoclaved in battery jars with water, almost any kind of tree being satisfactory. A piece of plain glass was used to cover the jar. In this material the fungus grows rapidly and may fill an 8-inch jar in an interval of 2 months or less.

Tree seedlings to be tested were grown in a screened mixture of sand and greenhouse soil in frames set into the ground, the inoculum being placed from 5 to 6 inches below the surface of the soil. In order to avoid root injury to the seedling through transplanting, seed was ordinarily used and was planted in the upper 2 inches. The peach pits were an exception; they had sprouted before planting, but the roots did not extend down more than 2 inches below the soil surface. This method eliminated, as far as possible, any root injury. Seedlings were dug for examination at various intervals, and occasionally one would show the stage desired. Persian (English) walnut (*Juglans regia*, Concord variety), northern California black walnut (*J. hindsii*), peach (*Prunus persica*), myrobalan (*P. cerasifera*), and French pear (*Pyrus communis*, Winter Nelis and Surprise varieties) comprised the list of seedlings used for study. All grew fairly well except the myrobalan, only a few of which came up and for some unknown reason these escaped infection. Myrobalans referred to later in this work were taken from another experiment in which 1-year-old seedlings were planted in 10-inch flowerpots for a resistance test and had grown there for 3 years. Carrots (*Daucus carota*), parsnips (*Pastinaca sativa*), dahlias (*Dahlia* sp.), and potatoes (*Solanum tuberosum*) were grown in boxes of inoculated soil much the same as the tree seedlings.

After the inoculum has been in the soil for several months, it will, on being dug and placed in a moist chamber, produce a large quantity of rhizomorphs in a period of 2 to 4 weeks (pl. 1, *B*), and if kept moist these rhizomorphs will attain considerable length. Objects like potato tubers may be brought into contact with the rhizomorphs and the invasion by the fungus watched. Such a method was used in some of the potato studies reported herein.

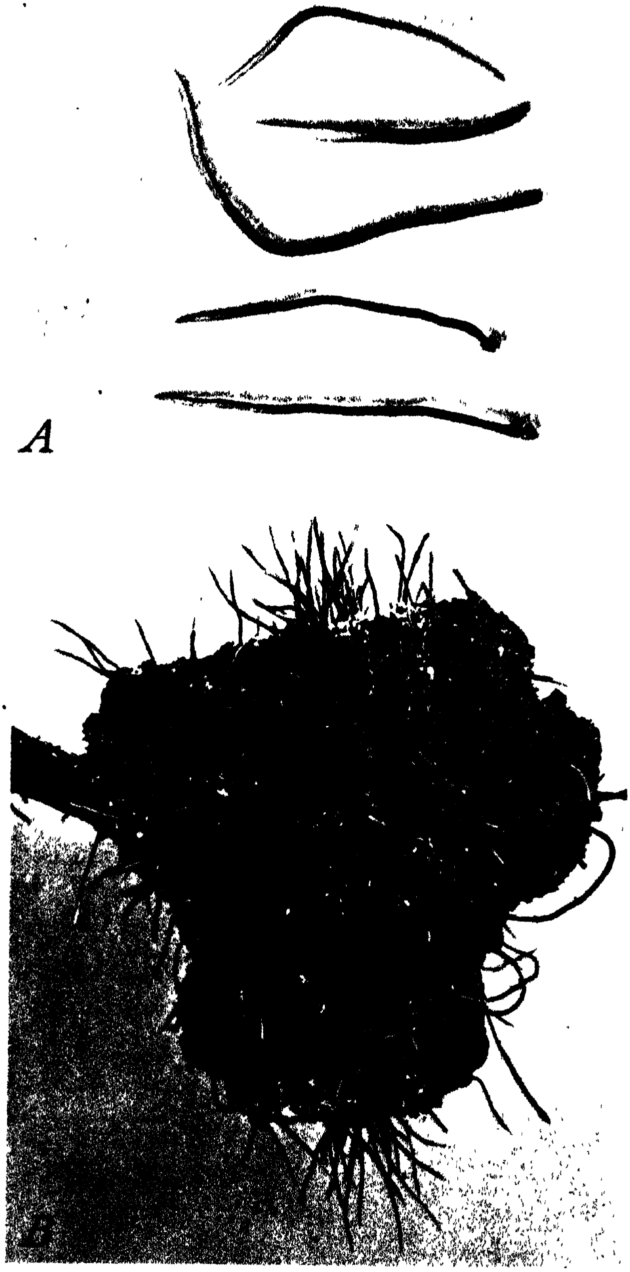
For killing and fixing agents, Rawlins' (*34*) alcohol-formalin-acetic formula and Flemming's weaker solution were used, and others tried. Flemming's weaker solution appeared to cause a little less shrinkage of the rhizomorph than did the others. Various stains were used during the study. Vaughan's (*40*) modification of Pianeze's IIIb stain, and Flemming's triple were used most extensively. The former proved to be the best, giving good differentiation of the fungus, the host, and the diseased tissue. Both paraffin and freehand sections were used throughout this study. With woody tissues the paraffin method is not ideal, the wood usually becoming too hard to section perfectly. The hydrofluoric acid treatment caused injury to the fungus and was not generally used. In order that the whole history of the lesion be known, its entire length was always sectioned. This proved definitely whether a wound or lenticel was originally present at the point of entrance.

Of the fleshy roots or tubers used for the infection study the potato, carrot, and parsnip were found to be susceptible, the potato being rather the most so. The dahlia tuber seemed to show more resistance but was not immune. Of the tree seedlings, field observations indicated that the Persian walnut and peach were very susceptible, the myrobalan had some resistance, while the French pear and black walnut were very resistant.

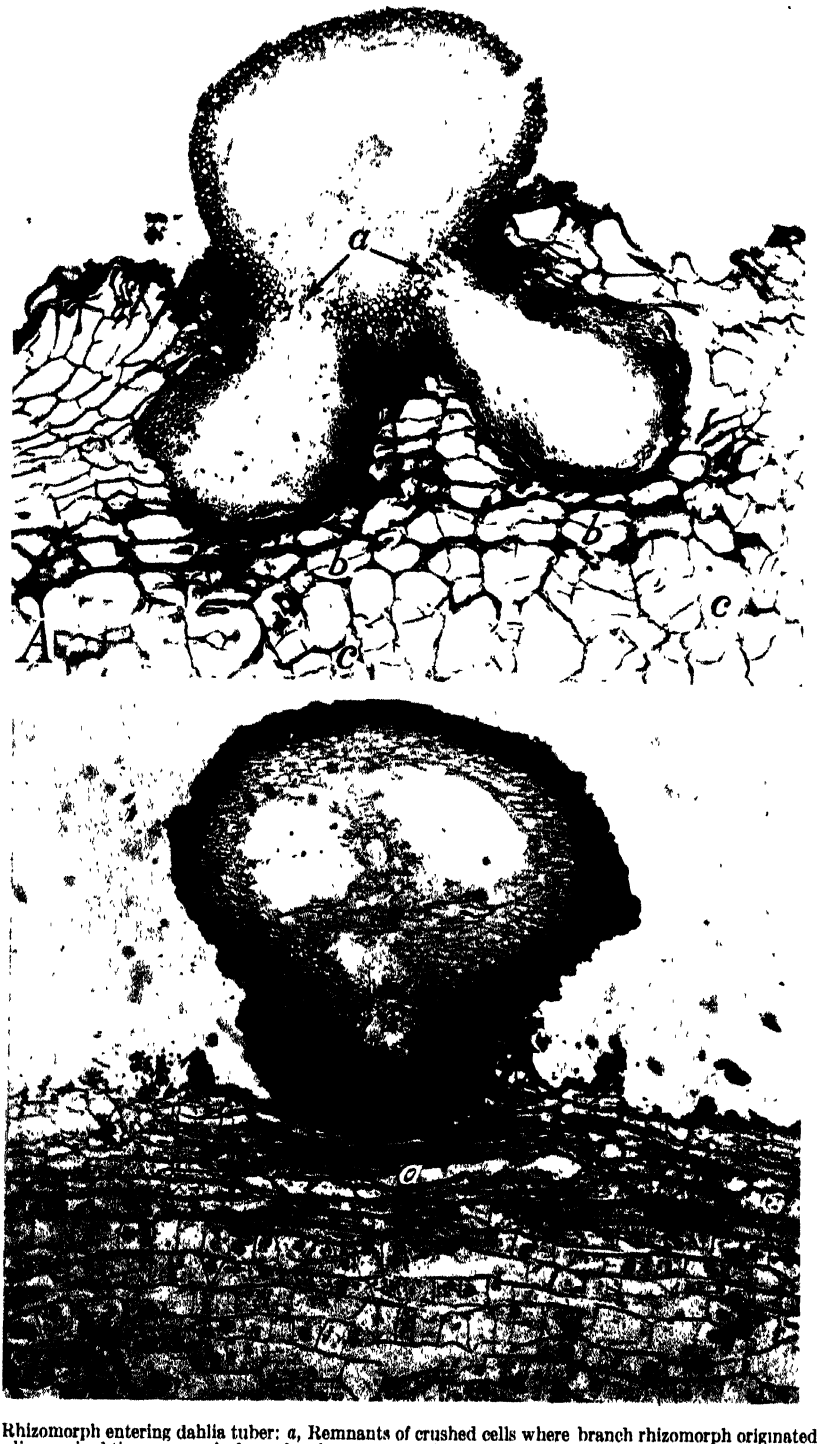
EXPERIMENTAL DATA

ATTACHMENT OF THE RHIZOMORPH

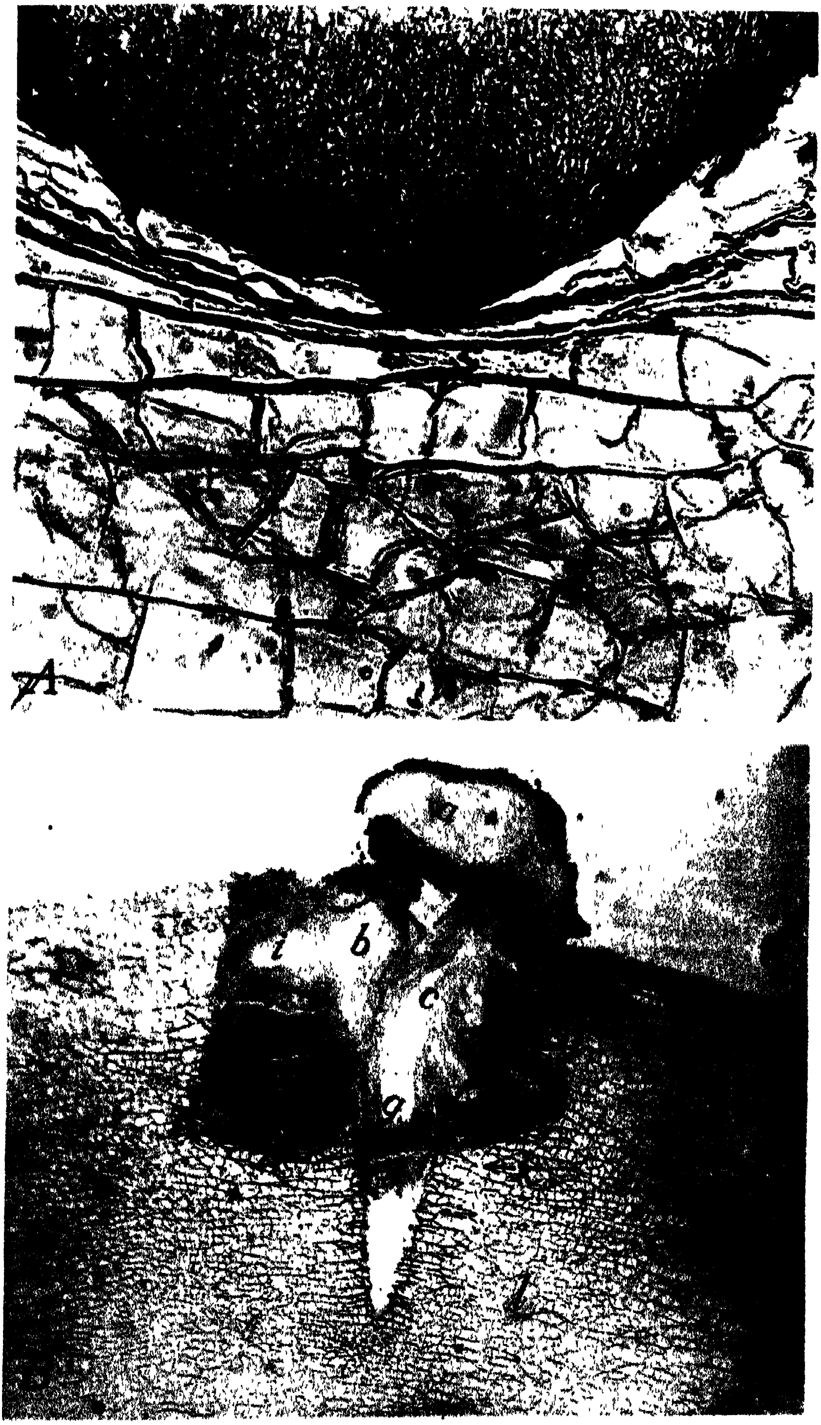
When the free end of the rhizomorph comes in contact with a root, it, in some cases, becomes rather firmly fixed. The attachment is brought about, partly at least, by the hardening of the mucilagi-



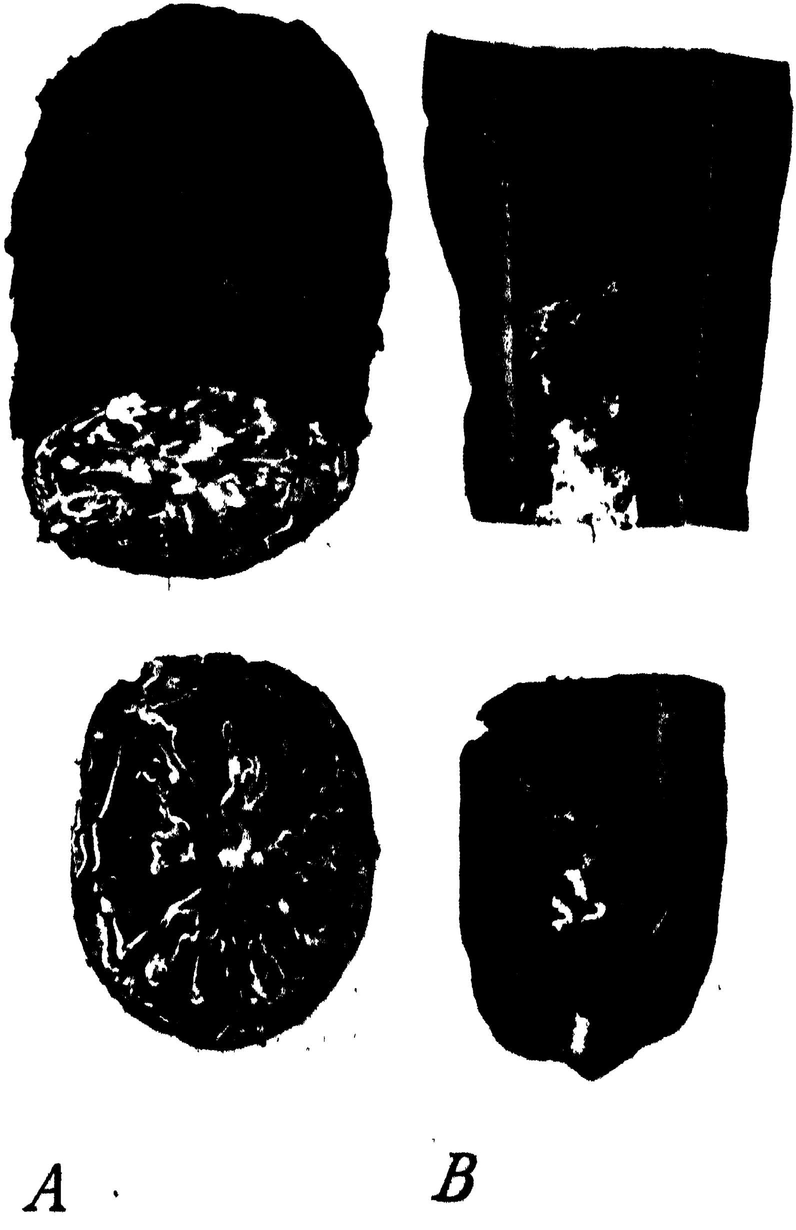
A, White tips of growing rhizomorphs. × 3. *B*, Rhizomorphs growing from a piece of inoculum in a moist chamber after having been in soil for 6 months. × 1.



A, Rhizomorph entering dahlia tuber: *a*, Remnants of crushed cells where branch rhizomorph originated; *b*, disorganized tissue; *c*, newly formed cork. × 90. *B*, Parsnip; first stages of entrance by a rhizomorph: *a*, Compressed and deeply staining tissue below invading rhizomorph. × 90.



A, Carrot; detail of the tip of an entering rhizomorph. × 240. *B*, Carrot, invading rhizomorphs beginning to branch: *a*, Parent rhizomorph; *b* and *c*, infection rhizomorphs, *d* to *i*, secondary branches; *j*, compressed and disorganized host tissue, *k*, affected but not disorganized tissue beyond *j*; *l*, normal tissue. × 22.



A, Carrot completely permeated by internal rhizomorphs. × 1. *B*, Rhizomorphs extending in pockets in the central cylinder of the carrot. × 1.

nous substance which envelops the rhizomorph close behind its white tip (pl. 1, *A* and *B*). This material, when dry, gives the shiny appearance to the surface of the rhizomorph, and when it dries in contact with the root surface helps in gluing the rhizomorph to the root. Single side hyphae developing from the rhizoromph tip and penetrating into the outer layer of cork cells act as anchors to hold the strand fast. In a moist chamber, on potatoes the rhizomorph has been observed to be attached rather firmly for an inch or more along the surface. Usually the attachment is not continuous, the rhizomorph being alternately loose and joined. The base of the white tip is sometimes firmly fastened and at other times free. This undoubtedly determines whether or not the rhizomorph is to become attached at that point, for after the mucilaginous substance has once hardened, there is no means by which the rhizomorph may become fastened.

DEVELOPMENT OF A BRANCH RHIZOMORPH

Branches normally develop at the point of contact of the attached rhizomorph and the root surface. They appear always to develop where the contact is very secure and never where the rhizomorph is unattached. They are to form the penetrating rhizomorphs (pl. 2, *A*). The branch originates in the inner cortical cells of the rhizomorph where some stimulus, thought by Brefeld (*6*) to be a contact stimulus, is set up causing the hyphae of the parent rhizomorph in this region to branch laterally. These hyphae then force their way through the outer cortical cells by tearing and crushing or otherwise destroying them and emerge as a branch. A few of the torn cell remnants often remain (pl. 2, *A*, *a*). Branches may be fairly numerous under an attached rhizomorph, apparently always developing on the side of the rhizomorph in contact with the host and seldom, if ever, from the opposite side. Their constant occurrence in the one position emphasizes the importance of the stimulus or stimuli initiating the process.

MANNER OF PENETRATION OF THE RHIZOMORPH

FLESHY ROOTS AND TUBERS

It has already been seen that the rhizomorph is apparently capable of attaching itself at any point on the root or tuber, and that branches form regularly where firm attachment has occurred. If infection now takes place, the branch must enter through the corky or suberized covering which protects the root or tuber. This must be done either by mechanical force, in which case the suberized cells would be either crushed or pushed aside, or by solvent action of the hyphae on the suberized layer with subsequent growth through the dissolved mass, or by the splitting apart of the cells which form the cork layer, at their middle lamellae, and entering in these splits. The manner of entrance into fleshy roots is similar in its general aspects for all those investigated. After entrance has once been gained, details of the effect on different roots vary slightly. The first stage of entrance is illustrated in plate 2, *B*, where the fungus is preparing to enter a parsnip. A detail at the tip of a rhizomorph is shown in plate 3, *A*, in which the initial stage of entrance into a carrot is illustrated. It is readily seen that the rhizomorph enters as a unit, that the hyphae composing the penetrating rhizomorph tip are acting en masse, and that there are no single threads extending beyond this mass.

Up to this stage entrance is by mechanical force, the host cells under the rhizomorph being pushed in and slightly compressed. There is no destruction of tissue and very little evidence, if any, that there is any solvent action upon the suberized layer and certainly no splitting of these cells at the middle lamellae with penetration through the splits. But it will be noticed in plate 2, *B*, that the walls of the cells at *a* under the two outer layers of cork cells are stained very deeply, while similar cells not in this region do not take this heavy stain. This gives evidence that these cells are not normal and may be affected by something given off from the tip of the penetrating rhizomorph. The tip of the invading branch continues to push in, finally penetrating through the suberized layer into the tissue below. There is distinct evidence that mechanical pressure is playing a part, since some of the collapsed cells surrounding the tip are pushed to the side.

The formation of secondary branches by the penetrating rhizomorph often occurs at the next stage of growth in a fleshy root or tuber. Plate 3, *B*, *d* to *i*, illustrates such branching in a carrot. This particular section shows the parent rhizomorph (*a*) on the surface of the carrot but the branching from it of the infection rhizomorphs (*b* and *c*) does not show at their point of emergence, which is demonstrated in a later section where the two branches originate at about the same place on the parent, one from either side. In the section shown, after penetration has occurred, the branches (*b* and *c*) appear almost as one. Each shows the start of two or more branches which will soon develop, and from here the spread in a susceptible host is fairly rapid. After a young branch (pl. 3, *B*, *d* to *i*) has attained a little more extension it sends out from near its base single hypae which spread into the surrounding tissue and continue the destruction of the host cellular structure. As the tip of the branch extends, these side hyphae follow, growing out perpendicularly to its surface. In an advancing rhizomorph they were never found to extend ahead of the tip, but in one no longer extending they would probably be found growing from the tip. The cells immediately surrounding the secondary branches, as well as the primary invading branch, are affected chemically; their walls are closely compressed and the whole so changed that they now present only an indistinguishable mass, which with Pianeze's stain takes a dark-green color indicating death and partial destruction of the cells (pl. 3, *B*, *j*). For two, three, or more cells deep around this mass the stain is not taken normally (*k*). The walls may become a pale yellowish green if they assume any color at all. In some hosts they are slightly brown and fail to take the stain. The cell contents, if such are present, assume an even paler green. Beyond this (*l*) the cells appear normal, and both wall and contents take a pink stain. Observed macroscopically from the surface and in the fresh state, at the stage indicated in plate 3, *B*, or a little later, there is found under the attached rhizomorph a brown area, at first small and pale brown but soon enlarging and darkening at the center. It indicates that the rhizomorph has entered and is acting parasitically on the host. Plate 4, *A*, illustrates a late stage in the destruction of the carrot.

Plate 2, *A*, illustrates a case soon after penetration has taken place in the dahlia, a somewhat resistant root. The branch of the rhizomorph has pushed through the cork layer intact down into the tissue under it and is affecting it chemically, as indicated by the different staining reaction at (*b*). Up to this point there has been no essential difference in detail between penetration into the susceptible or resistant roots, but now a difference is observable. Under the mass of affected cells in the resistant dahlia there has been formed by the host a layer of cork, which walls off from the remaining part of the tuber the disorganized area surrounding the entering rhizomorph, and further development of the latter appears stopped. In plate 2, *A*, this layer of cork (*c*) appears as a line of dividing cells, but when stained with Sudan III it gives the typical test for suberized walls. The development of this suberized layer around the infection branch is a rather constant response of this host to invasion by the fungus. Of 13 cases examined each showed a corky layer, or dividing cells preparatory to its formation, soon after entrance into the tuber had been effected. In the susceptible carrot and parsnip an imperfect line of dividing cells is occasionally found, but a definite layer of suberized cells is seldom formed. In the potato, the most susceptible of the fleshy roots or tubers studied, no case was found where dividing cells were forming. The significance of the cork layer will be discussed in a later paragraph.

SUSCEPTIBLE WOODY ROOTS

The hardening of the woody tissue during dehydration makes it almost impossible to cut woody sections without some tearing by the knife, especially of the bast fibers and hard lignified walls. Since only the first stages of entrance were of importance at this time, in most instances the bark only was removed and sectioned, there being no need to study the wood below. The bark alone cut more easily than did the bark and wood together. The Persian walnut proved to be exceptionally good material for this study. It has a more fleshy root than the peach and cuts more easily.

The first stage of invasion is represented in plate 5, *A*, where a small infection branch (*a*) is penetrating through the cork tissue. The parent rhizomorph is not firmly attached to the host in this section, probably having been torn away during sectioning or previous handling.

A slightly later stage is illustrated in plate 5, *B*. The rhizomorph is expanding laterally in the cork tissue, destroying some of the suberized cells but here extending mainly by mechanical force. Chemical action is, however, not absent, as indicated by the disorganization of the tissue below the remaining 2 or 3 layers of cork cells at *a*. This section is through the approximate center of the entering rhizomorph and shows the deepest point to which the rhizomorph has penetrated. It gradually recedes as sections on either side are examined, leaving little doubt that the influence felt below the cork is ascribable to secretions of some sort from the rhizomorph diffusing through the remaining 2 or 3 layers of cork cells at *b*.

Plate 5, *A*, also shows considerable disorganization under the penetrating tip, but this is attributed, at least in part, to an adjoining deeper infection at a short distance from this one, with its influence extending out below the one illustrated. As in the case of fleshy roots, the infecting rhizomorph is penetrating as a unit with no indication of single hyphae radiating out in advance.

At the next step the rhizomorph pushes through the remaining cork and down into the tissue below, where branching usually takes place; its progress is essentially the same as in the fleshy roots previously described. In a young walnut seedling, as used in this study, the large tap root is very fleshy, being somewhat like a carrot in shape with a tapering shoulder at the top. The cortex is largely parenchyma tissue, enclosing a thin woody cylinder surrounding a large pith. The internal rhizomorphs of the fungus readily permeate the parenchyma of the cortex, quite the same as in the carrot or parsnip, and then grow through the thin woody cylinder into the pith where the growth is very rapid and may outstrip that in the cortex. The growth is so luxuriant that the pressure exerted by the rhizomorphs as they expand in the pith is sufficient at times to split the main root of the small seedling longitudinally.

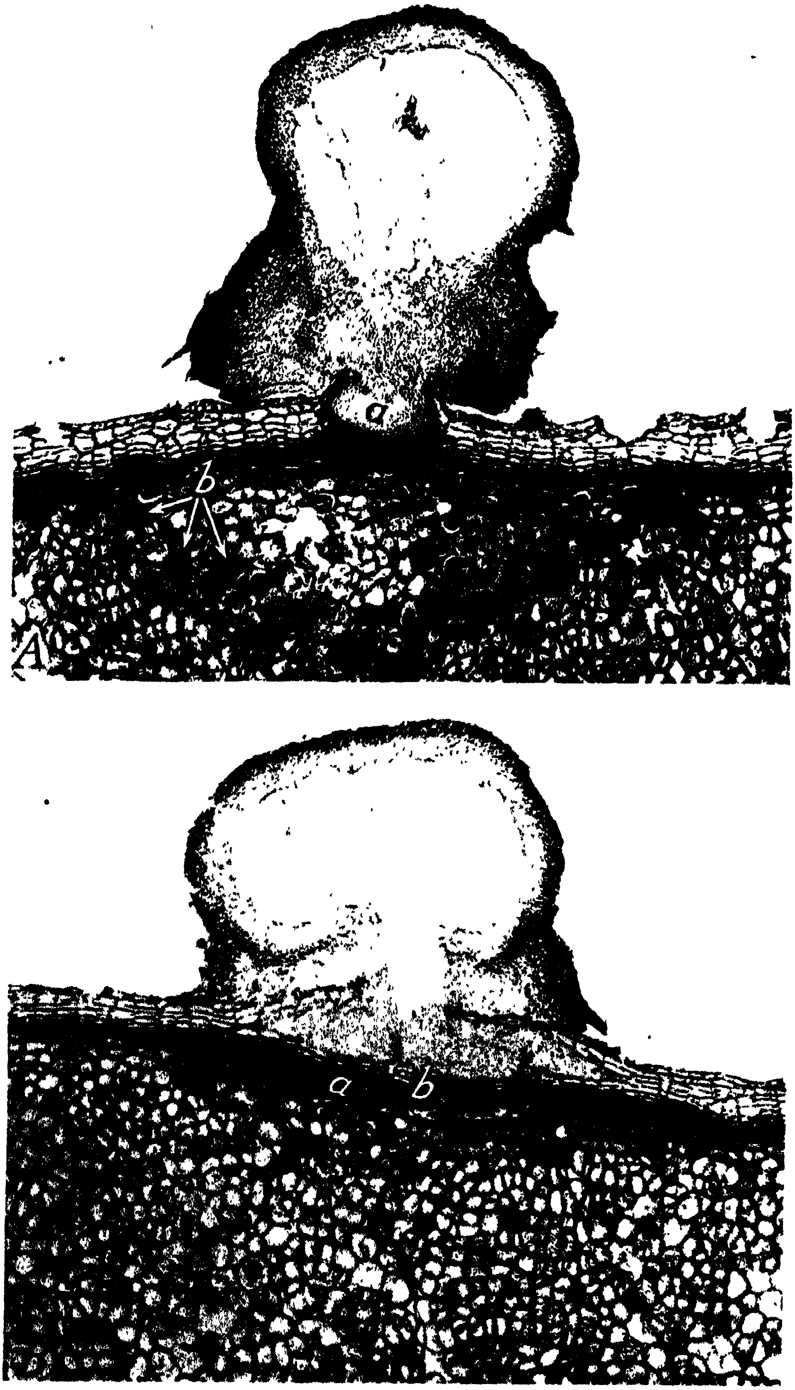
It was pointed out earlier that the rhizomorph may attach itself to the root for some distance and form infection branches at several points close together. This was well illustrated in a walnut root. A rhizomorph was attached rather firmly for a distance of 1¼ inches. Sectioning of this entire length of root revealed the fact that a branch of the rhizomorph had either entered or formed preparatory to entry at each of 15 separate points.

Of the many walnut roots examined in this study at least two were found in which entrance had been gained at a lenticel, but in the majority of cases entrance was through the apparently uninjured and unaffected cork layer of the root.

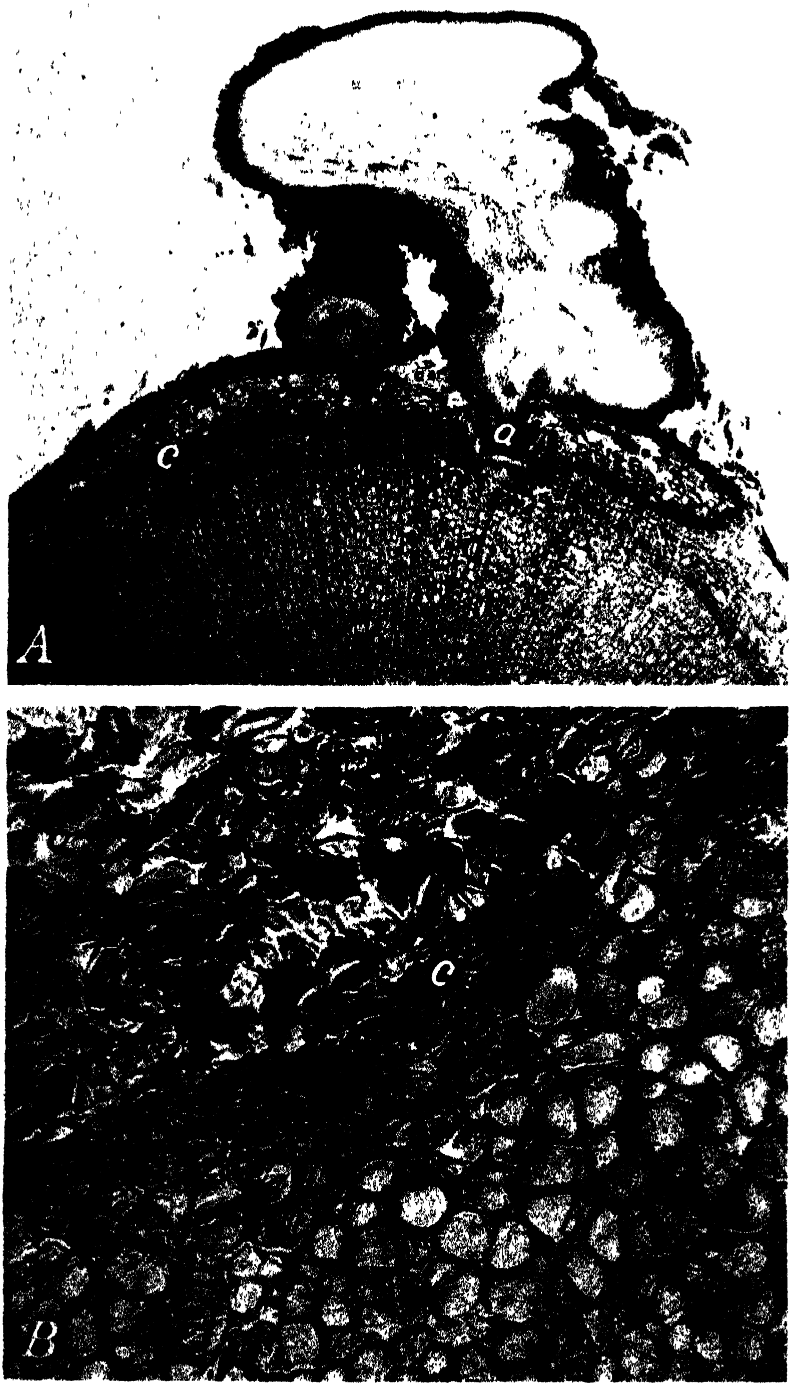
Although the roots of 20 or more seedling peaches were found infected at one or more places, it so happened that none was found at the time when actual penetration by the rhizomorph was taking place. Later stages of the infection, however, did not indicate that penetration was different in this root from those heretofore described. One feature of growth in the peach root which had not been noticed in any of those previously studied was the formation of cavities or pockets, filled with gum, in the roots or main stem, a short distance beyond the point where the internal rhizomorph was advancing. These cavities were always close to the cambium layer, sometimes just under it, at other times including the young wood and the cambium and a part of the phloem. They were always situated between the medullary rays, the latter seldom, if ever, being included in the cavity.

RESISTANT WOODY ROOTS

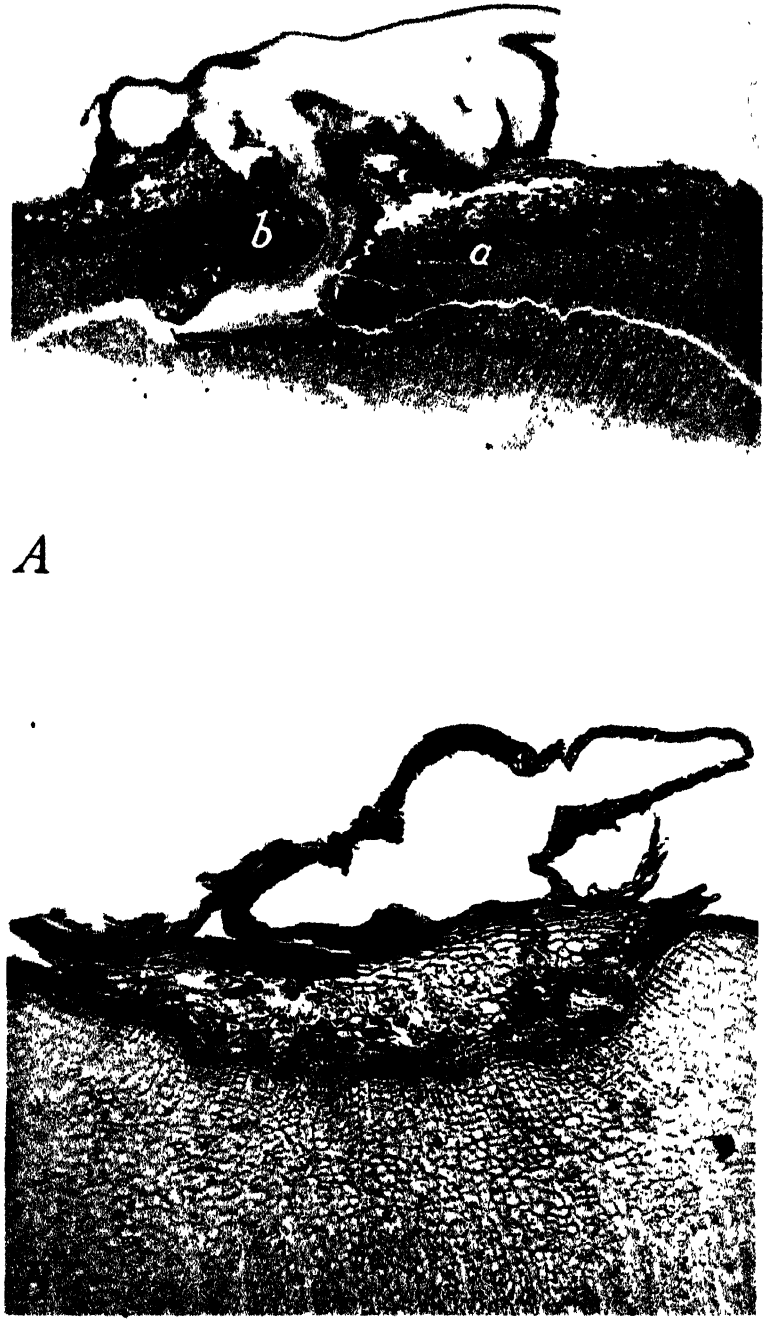
Seedlings grown from seed of the French pear, varieties Winter Nelis and Surprise, were used as resistant roots for this study. They were dug and examined from time to time over a period of 9 months to determine the mode of entrance of the fungus and the host reactions. The first stages of penetration into healthy pear tissue indicated that entrance was directly through the sound cork, just



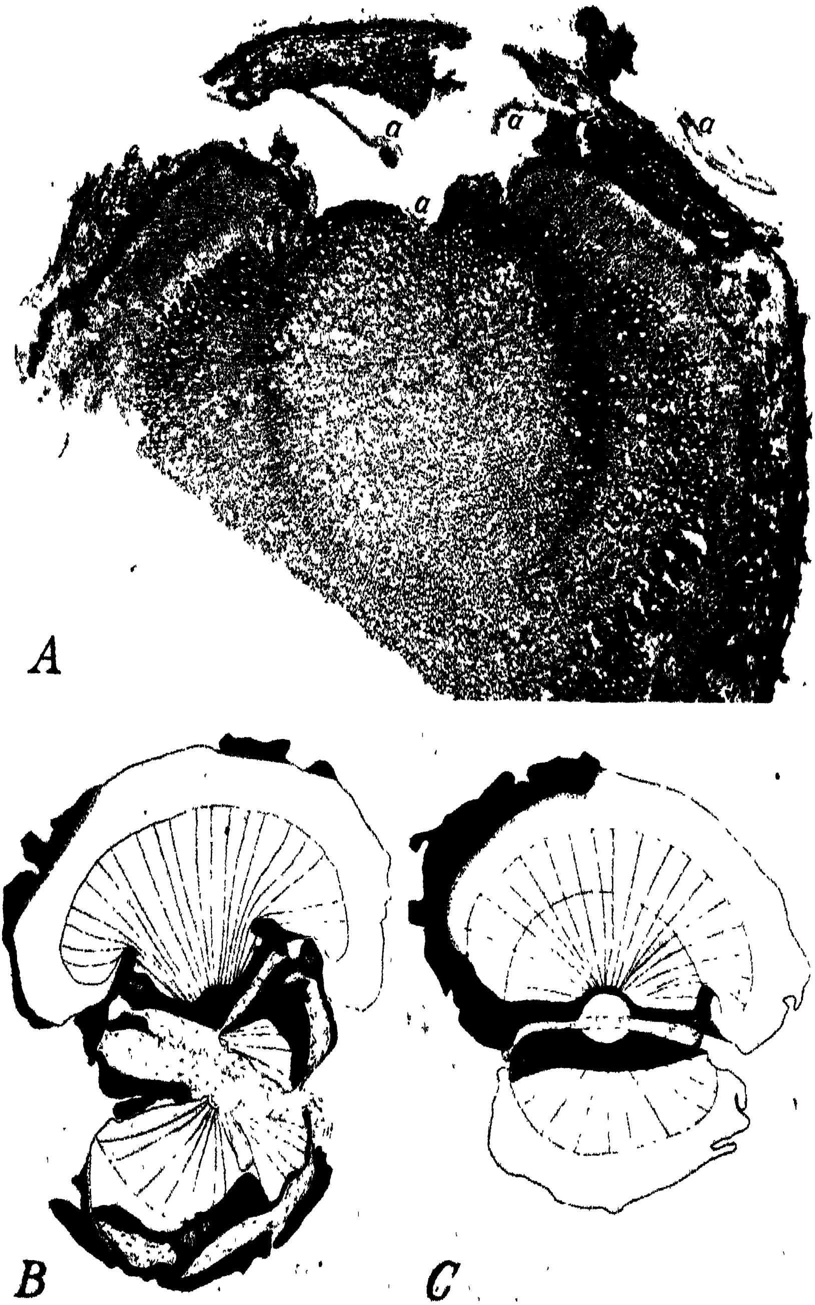
A, Persian walnut root (susceptible); first stages of penetration by a branch rhizomorph *a*, Infection branch; *b*, deep staining deposit filling cells at edge of lesion × 62. *B*, Persian walnut root; rhizomorph spreading laterally in the cork layer causing some compression and destruction of the cork cells *a*, Disorganized tissue; *b*, a layer of cork 2 and 3 cells thick remaining between the rhizomorph branch and the disorganized tissue. × 62.



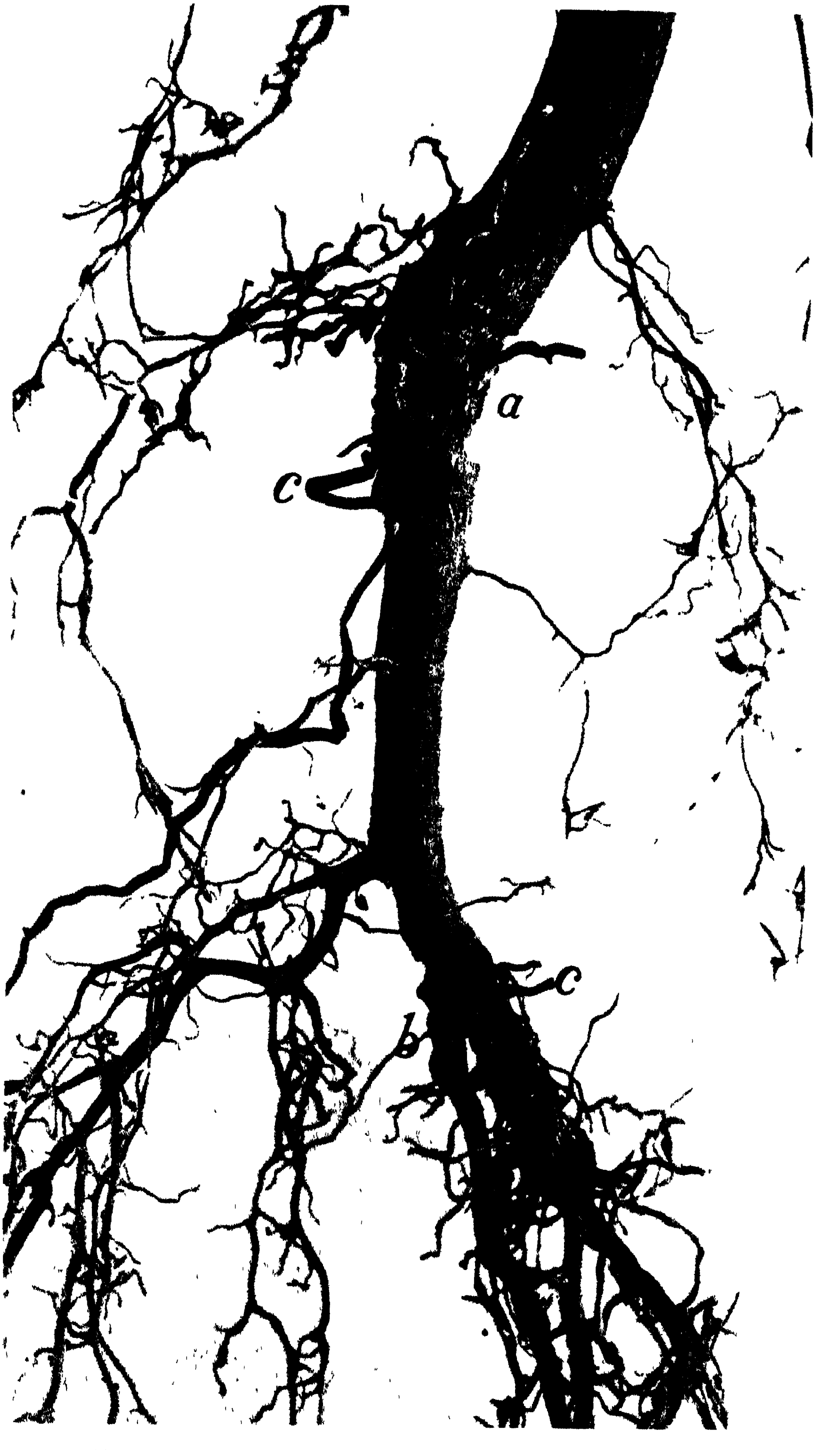
A, Pear root; entering rhizomorph: *a*, Extending below the cambium; *b*, a branch entering original cork layer; *c*, secondary periderm; *d*, affected wood. × 43. *B*, Pear root; detail of secondary periderm. *a*, Disorganized tissue inside lesion; *b*, normal tissue outside lesion; *c*, phellogen × 230.



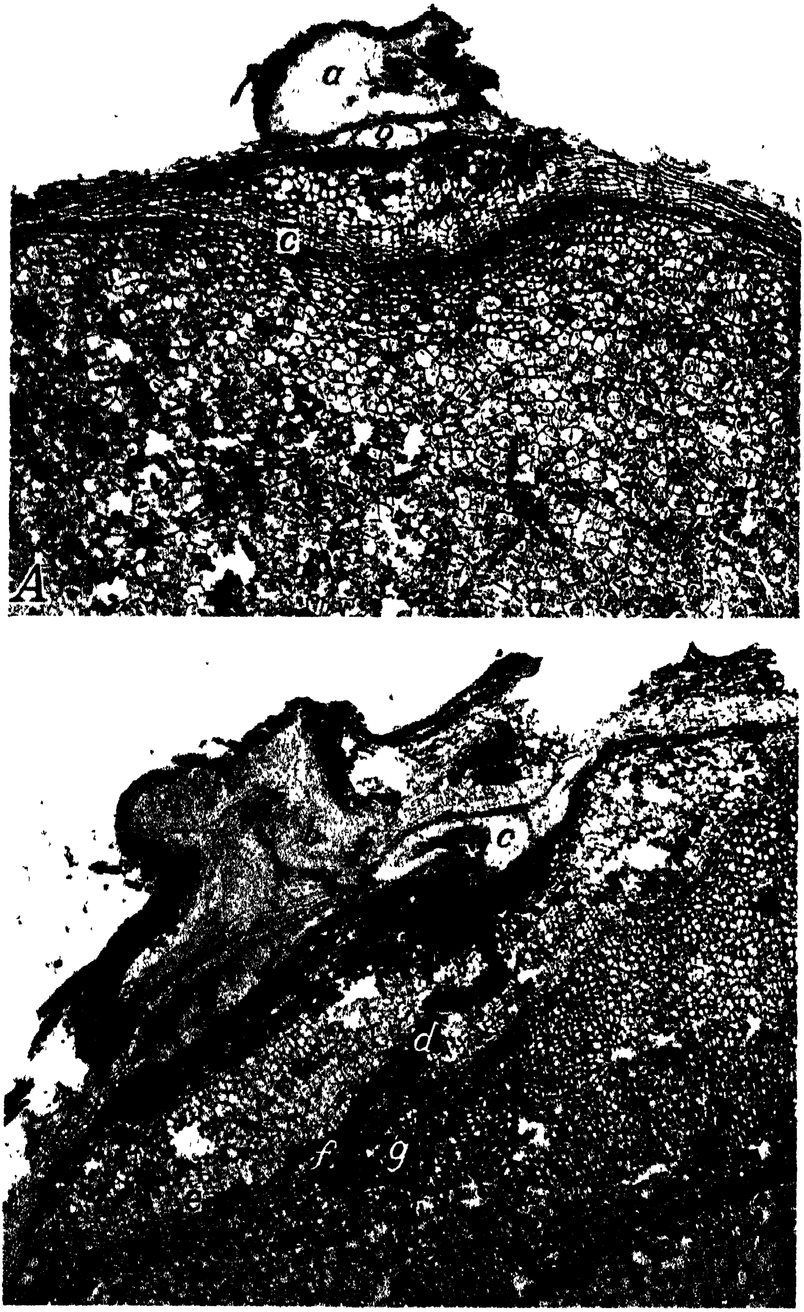
A, Pear root; rhizomorph entering wood below cambium *a*, New cork, *b*, crushed cells. × 22. *B*, Pear root; rhizomorph failed to penetrate below the original cork; the wound has been walled off by a layer of secondary cork. × 62.



A, Pear root; an old deep lesion healing out: *a*, Remnants of old rhizomorph. × 24. *B*, Pear root, camera lucida drawing of a cross section of an affected tap root at approximate position where rhizomorph entered *C*, Below *B* in the same lesion; crosshatching indicates the fungus; the very dark area the dead, browned wood or cortex; the lighter areas the less affected wood; and the uncolored areas the normal healthy tissue. × 11.



Pear root; *armillaria* lesions at *a* and *b* The root is affected over its entire circumference at *a*, *c*, rhizomorphs. × 3.



A, Black walnut root; old rhizomorph on surface walled off by new cork: *a*, Old rhizomorph; *b*, branch rhizomorph which has penetrated original cork; *c*, new cork. × 38. *B*, Black walnut root, a rhizomorph with several branches, one of which is penetrating the secondary periderm. *a*, Original rhizomorph, *b*, primary branches, *c* and *d*, secondary branches; *e*, new periderm partially penetrated at point *f* by the branch rhizomorph *d*, *g*, disorganization in tissue at this point caused by rhizomorph branch *d* before completely penetrating through periderm *e*. × 28.

as in the susceptible walnut root. In plate 6, *A*, the rhizomorph has entered at *a* and is entering at *b*, but we are hardly justified in considering this latter to be entering sound tissue, for the entrance of the first rhizomorph has undoubtedly caused some disturbance in the cells under the second. When the invasion of the first rhizomorph took place some host reaction occurred, causing the root to start the formation of a periderm in the cortex tissue below. This shows as a dark line in plate 6, *A*, *c*. On the lesion side of this line, the cells are much disorganized (pl. 6, *B*, *a*), whereas on the other they appear quite normal (pl. 6, *B*, *b*), indicating that the influence from the rhizomorph, whatever it may be, has been checked at this line. It is plainly evident that the rhizomorph in plate 6, *A*, *a*, has not been checked in its advance by the formation of the periderm. It has broken through this layer and through the cambium and is causing some disorganization of the cells in the wood below, as evidenced by the deeper staining area at *d*. Another instance in which this secondary cork layer has been penetrated is illustrated in plate 7, *A*. The section has been badly broken in cutting, but the important features stand out clearly. The rhizomorph has broken through the young cork (*a*) and through the cambium and is growing in the wood. That some mechanical pressure is exerted is evidenced by the crushing of the cells above the rhizomorph in the region of the cambium at *b*, although this does not show clearly in the photomicrograph.

The rhizomorph does not in all instances break through the secondary cork layer in the manner illustrated in the foregoing cases. In plate 7, *B*, is shown an instance in which the rhizomorph failed to enter further than the primary cork layer. The lesion produced has been definitely walled off by the secondary periderm. This cork layer widens with the growth of the root, but any sloughing of its outer cells is prevented by the tissue forming the lesion, which remains intact on the surface. The loss of the outer cork cells surrounding the lesion finally leads to a situation where the old lesion is clinging to the surface of the perfectly normal appearing root. With the continuation of root growth it is finally lost.

In plate 8, *A*, a few remnants of an old rhizomorph (*a*) remain at the center of the lesion, enough to show the probable cause of the injury. The parasitism of the fungus has been overcome by the host and the latter is now attempting to heal the wound with growth from the cambium. Plate 8, *B*, illustrates a very severe lesion in the tap root of a small Surprise pear seedling. This section, made at about the place where entry occurred, shows the destruction which may be caused in the host and yet not kill the root. The internal rhizomorphs have split the root in half, almost completely killing one half and invading a part of the other, yet at this time the host has apparently overcome the fungus and is beginning to heal over the wound by cambial growth. Plate 8, *C*, illustrates a section lower down in the same lesion where the root is split but less affected on the two sides. The fungus is making no headway other than the splitting of the root, and healing is progressing. Many similar instances of this general effect were observed, especially in later examinations after the early infection had ceased to be active. A surface view of such a lesion is shown in plate 9.

From data presented thus far it will be seen that resistance in the pear can hardly be accounted for by the failure of the rhizomorph to enter the host. Nor does it appear that any morphological obstructions are present and responsible for resistance. In this host the fungus readily enters and may penetrate to the cambium and into the wood below, at times killing tissue deep in an individual root. It might be expected that the fungus would now be free to penetrate any portion of the root. A wound is established, and it could act as a wound parasite. But in the pear it fails to develop further and kill the tree and seems to have very little effect upon the tree other than at the point where the lesion occurs. In this experiment no cases of pear trees clearly showing abnormal yellowing or wilting of the top were observed. Of the hundred or more trees in each lot an occasional tree died, but these were usually the small inferior ones, badly crowded; there was no certainty that death was due to *Armillaria*, although the fungus was sometimes found in the roots. It may enter any dead root. In contrast, when the susceptible Persian walnut and peach are entered no such reaction occurs. The fungus on reaching the cambium, or before, spreads rapidly, usually girdling the root and eventually causing death to the top.

The black walnut represents a species exhibiting considerable resistance in the field and has long been used as a resistant stock. Seed gathered from an isolated tree was planted in inoculated soil and the seedlings were dug from time to time. Lesions appearing on the roots were sectioned, and the penetration of the rhizomorph was studied in 14 separate infections where the fungus had penetrated through the root periderm. Of this number 12 had been checked and walled off by cork. The remaining 2 infections became deep and, at the time collected, had not been walled off by cork, although there was some indication in 1 that the spread had been arrested. In the latter, at about the usual position where cork formed in the others, there was found a line of cells the contents of which took the stain deeply and showed evidence of disintegration. While a high percentage of the infections in the seedlings examined had been checked at the first stage of infection, i. e., after the act of penetration, it does not mean that all are stopped at this stage, for many of the seedlings that were left in the inoculated soil eventually died and, on digging, were found to have their roots thoroughly permeated by the internal rhizomorphs of the fungus.

The essential features concerned with the penetration of the rhizomorph into the black walnut root vary but little from the manner in which the resistant pear root is invaded. In plate 10, *A*, is illustrated the very early checking of the penetrating rhizomorph and the subsequent formation of cork walling off the area. In this instance the rhizomorph penetrated only through the cork and was then stopped. The influence of the rhizomorph has extended a few cells beyond, as evidenced by the darker staining. Plate 10, *B*, illustrates another instance in which the rhizomorph has been checked and the affected area walled off by cork, but in this case a branch rhizomorph (*d*) is penetrating the secondary cork layer and is doing so in much the same manner as that illustrated in the pear (pls. 6, *A*, and 7, *A*). The rhizomorph is not entirely through the cork, but the cells in the tissue under it are plasmolized and darkly stained, indicating the effect of some diffusible substance from the rhizomorph. The two lesions illustrated above are typical of those examined. In some the rhizomorph penetrated deeper and invaded more of the bark, but in only one instance did it appear that the infection was spreading. It must be admitted in this connection that only the smaller lesions were examined. In the larger lesions the fungus was undoubtedly making headway, otherwise some of the seedlings would not have been eventually killed. The essential difference between the infection of the susceptible Persian walnut root and the resistant black is that very few infections fail to become established in the former or become checked and corked out, whereas in the latter it is the rule rather than the exception that the rhizomorph is held in check and walled off by cork. Of the 14 infections comprising the study on the black walnut root in no case did the fungus enter at a branch root or at a lenticel. In two lesions the rhizomorph had penetrated the cork close to a small branch root, but there was no relation between the ruptured tissue around the root and the entrance of the fungus.

In the field the myrobalan is intermediate in resistance between the pear and peach. Pot experiments indicate that it has considerable resistance. Of 45 trees planted in pots only a very few actually died from *Armillaria*. Examination of the roots at various times after planting indicated that infection had occurred in practically all cases examined, and not only in one place on the root but usually in several. In one carefully examined root system 21 separate infections were noted. These surface lesions varied greatly in extent, ranging from as small as one fourth inch in diameter up to the entire circumference of a branch root. The tree was not dead and did not appear to be suffering greatly from the numerous disease lesions in the root. A more careful examination of the lesions indicated that the fungus was extending only very slowly in them, in some probably not at all. The borders of the lesions consisted of tissue which had turned a bright red and was extremely hard and brittle. It is possible that this layer, on account of its hardness, is in part responsible for the reduced activity of the fungus rhizomorph in these tissues, but more fundamental reactions must occur in the host before this layer is produced. The red zone is sometimes surrounded by cork but more often not. In the myrobalan the extent of the fungus in the woody cylinder of the root is often equal to that in the cortex. This differs from a susceptible host where the growth in the cortex and cambium is normally much more rapid than that in the wood.

CYTOLOGICAL ASPECTS OF INFECTION

Destruction of Cork

The studies thus far have given a general account of the histological features concerned with penetration and infection by the rhizomorph. More detail of some of the cellular phenomena will now be presented.

The dense mass of active hyphae composing the invading branch presses against the cortex of the root. At first, so far as staining reactions indicate, it has very little chemical effect upon the walls of the cork layer. As the pressure continues, however, the suberized walls that are in direct contact with the active rhizomorph tip seem to disappear as if acted upon by some dissolving force. They undergo little, if any, swelling, and this only in the very latest stage before their complete disappearance. There is no general breakdown of the cork tissue for any distance away from the rhizomorph. The latter acts on and destroys only those cells with which it comes in contact and against which it exerts pressure. The rhizomorph may grow laterally in the cork and split it apart longitudinally (pl. 5, *B*) after once penetrating into it, but many of the cells that originally occupied the area where the rhizomorph has actually pushed through have disappeared and no remnants of them remain. Neither are they engulfed and then destroyed. If mechanical pressure alone were responsible for penetration and no solvent action took place, one would expect to find the cork cells heaped up or folded back around the point of entrance. This has never been observed to an extent sufficient to account for all the cells originally present. As the cork under the invading branch of the rhizomorph is gradually acted upon and disappears, the latter pushes through into the cells below.

EFFECT OF THE RHIZOMORPH ON THE PARENCHYMA BEFORE INVASION

In certain of the hosts examined while the rhizomorph is in the process of entry, but before it has reached below the cork, certain reactions have taken place in the parenchyma directly under the penetrating rhizomorph which indicate that the influence of the rhizomorph is felt in the tissue below it. When the rhizomorph has penetrated into the second or third layer of the cork covering of the parsnip the parenchyma cells immediately under the rhizomorph tip take the red stain very deeply, when Flemming's triple is used (pl. 2, *B*, *a*). The cell contents stain somewhat deeper than normally, but the most noticeable effect is in the cell walls and the nuclei which stain much deeper than normally and indicate some influence of the rhizomorph. The walnut root often shows a very different reaction, from that described above, when the rhizomorph tip is still two or three cell layers away. The affected walls of the parenchyma directly under the cork, instead of taking an abnormal deep red as was the case in the parsnip, fail to take any stain at all and remain a light brown similar to that before any treatment. The cell contents are somewhat plasomolyzed and take the stain most deeply, becoming almost black in color. They are in striking contrast to normal cells with deep-red walls and lighter red contents. The fact that the cork cells in immediate contact with the advancing rhizomorph tip show so little disturbance possibly indicates their extreme resistance to fungal action in comparison with the cells just under the cork layer. There would seem to be little doubt that some substance of a toxic nature is given off by the invading rhizomorph tip which causes the apparent rapid death and chemical change of the cells below the cork layer. Yet, so far as the stains employed in this work indicate, this substance has comparatively little effect on the cork cells through which it must pass and where it would supposedly be more concentrated.

The lateral spread of this material is plainly more rapid in the tissue immediately under the cork than its depth of penetration into the tissue below. In plate 5, *B*, (walnut root) the extent of the disorganization is plainly visible. The disorganized cells extend laterally for five or six cell lengths beyond the ends of the penetrating branch, while at the center of this branch the downward extent is only three or four cells in their short axis. Other cases in the walnut were even more pronounced than this one. No evidence was found that toxic action to the host occurs under an attached rhizomorph which is not preparing to enter.

EFFECT OF THE ADVANCING RHIZOMORPH ON THE TISSUE SURROUNDING IT

After the rhizomorph passes the cork layer, it enters dead disorganized tissue and never penetrates ahead of the dead cells. The nature of this killing action has not been investigated. The distance to which it may extend beyond the rhizomorph tip varies with the host and type of tissue. The parsnip presents an exceptional case in that there is a network of large intercellular spaces to be found in the fleshy cortical tissue. The toxic action has been observed to extend as much as 5.5 mm beyond the invading rhizomorph tip. It appears that the toxic material, whatever its nature may be, follows the intercellular spaces in the parsnip instead of passing directly through each cell to the adjoining one as appears to be the case in woody tissues. In the latter the action may extend only a few cells away from the rhizomorph.

The potato tuber presents a type of tissue upon which the fungus is very active. There is a marked killing and browning of the tissue around the rhizomorph tip. This varies in extent but often involves cells several cell widths distant. Kusano (*25*) indicates that the cell sap is the part which becomes brown. Observed macroscopically this would appear to be true, but the investigations here reported do not support such a view. The observed browning is due to the formation of a granular mass which first forms in the small intercellular spaces where the cells meet. It is next observed to occur along the inside of the cell wall, where it at first forces the plasma membrane away. Later the membrane disappears, and the brown material completely fills the cell cavity and often becomes less dense. It has much the same staining properties as the cell wall, namely, a lack of affinity for any stain. These facts suggest that this material may be a decomposition product of the cell wall. The protoplasts of the less affected cells take the stain normally but when stained are severely plasmolyzed. This is in contrast to the surrounding normal tissue. The disappearance of starch from the affected area appears to follow no definite rule. It is sometimes removed from the cells at the time of appearance of the brown material. Other times it remains until the individual side hyphae have penetrated into the area. In the very late stages of decomposition and just before their total destruction, the cells immediately surrounding the rhizomorph collapse completely. Their walls fold inward and are pressed closely together by the mechanical force of the advancing rhizomorph. The folded remnants of the protoplast lie between these swollen walls of indefinite outline. The mass assumes a very dark stain and then disappears entirely as the fungus rhizomorph grows against and into it.

While the growth of the fungus in the carrot is the same in its essential features as in the potato, the extent of diseased tissue away from the penetrating rhizomorph appears to be less and ordinarily extends but a few cells, perhaps indicating that the fungus is less parasitic on the carrot than on the potato. Gross observations lend some support to this belief. Once the potato has been penetrated, the fungus usually spreads rapidly and in all directions. In the carrot the spread may not be so extensive, as illustrated in plate 4, *B*, where the growth is slow and upon close examination shows the rhizomorph to be following small cavities or pockets in the central cylinder. The occurrence of these cavities is quite constant in the carrot but has never been observed in other hosts. On sectioning they appear as hollow spaces where the tissue has collapsed and drawn to one side. The relation of the cavity to the rhizomorph has not been fully determined. It appears always to connect at some point with the rhizomorph, but the latter does not ordinarily extend into the bulk of the pocket as might be expected if products from the fungus caused the collapse of the tissue.

The most noticeable effect of the fungus on the susceptible Persian walnut root is the rapid and complete browning of the tissue surrounding the rhizomorph. The cell walls are but little, if at all, swollen. They are definitely browned and have no affinity for any of the stains employed. No granular material collects along the walls as happens in the potato tuber. The original cell contents in the walnut root usually turn brown along with the wall or soon after and, like the wall, do not take the stain. Some of the cells fill with a very dense and darkly staining material, which, at least after being stained, is badly plasmolyzed (pl. 5, *A*, *b*). The formation of this substance inside the cell, along with the browning of the wall, is usually the first indication that the influence from the rhizomorph is felt below the cork.

A cell reaction, probably similar in effect to that described above when the walnut is invaded, is to be found in the myrobalan root. It is confined principally to that zone of red tissue that, in the myrobalan, usually borders a lesion produced by an invading or advancing rhizomorph, such as was described above under histology of infection. The occurrence and disappearance of the red zone may be followed best by examining unstained freehand sections cut through the border of the lesion, including the healthy tissue above and the disintegrated tissue close to the rhizomorph below. In such a section the live, healthy tissue entirely outside the lesion appears normal. The first indication of disturbance at the outer margin of the affected area is a slight yellowing of the cell walls in a region only a few cells in width. The contents of these cells, if at all changed, become less dense than normal. Then occurs a narrow zone in which the lumina of the cells are beginning to fill with a granular, colorless material, not dense at first but soon becoming so, and with this the red color develops, becoming more intense with the increase in density. This red zone often involves a region of considerable width. The red stain is entirely in the dense deposit filling the cell, which may normally occupy the entire lumen but under some conditions shows plasmolysis. The walls remain yellow as at first or darken slightly but never become red like the cell contents. The red fades out as the rhizomorph is approached, and the tissue becomes a yellow-reddish brown, the cell contents becoming less dense and finally disappearing and the whole mass showing signs of disintegration. The regions above described may vary appreciably in extent and at times may not be so clearly defined as indicated, but the red zone appears never to be missing. When staining with Flemming's triple, a somewhat different picture is presented. The dense cell contents, whether colored or uncolored, shrink into a small mass at the center of the cell, staining deeply. The walls in this region do not take the stain and remain a light brown or yellow. The zone close to the rhizomorph assumes a rather deep-reddish stain characteristic of disorganizing tissue.

The red zone is not confined to the cortex tissue, but the same material may be deposited in the cells of the wood as well, although here the intensity of the red is sometimes lessened and more irregular in extent. When tested for lignin with Maule's permanganate test (Morrow (*29*)), the walls in the red zone and below take on an amber color while the normal walls above stain a very deep pink, indicating the delignification of the cell wall by the action of the fungus.

The effect of parasitism on the above-described roots differs in at least one respect from that on the resistant French pear. This difference manifests itself in the lack of visible effect upon the cell walls of the cortex of the pear root when invasion occurs. They show none of the browning or yellowing and have no affinity for stains which is so characteristic of affected walls in the other hosts studied and which was one of the first observable symptoms of parasitism in the Persian walnut, always preceding the growth of the rhizomorph into the tissue. That region of tissue in other woody hosts with brown walls and deep-staining granular contents, which usually precedes the narrow zone of more completely disorganized and deep-staining cell material in direct contact with the rhizomorph, appears to be lacking in the cortical tissue of the pear, and only the disorganized zone extending but a few cell widths away from the invading rhizomorph is present. In the young pear roots examined this contrast was quite evident.

WOUND GUM

To all appearances the red, deep-staining substance which is deposited in the cells of the zone of affected tissue in the myrobalan is similar to that occurring in the walnut and might properly be termed "wound gum." Its formation and location with respect to the diseased tissue closely resembles the wound gum as described by White (*44*) when *Fomes applanatus* is acting parasitically upon various forest trees. He describes it in the beech as causing a band of dark-colored material which creeps forward as the fungus advances into the wood. It forms only in the newly attacked cells and disappears as the fungus advances, leaving no trace behind. The gum is described by others but there is some disagreement as to its origin. Tschirch (*39*) believes it to be a secretion of the living protoplasm bordering a wounded area. Münch (*30*) maintains that it is an oxidation product of the cell contents forming after their death. White (*44*) finds tyloses with the wound gum and considers that they can only be produced from living tissue and consequently "if wound gum is not a secretion it follows very closely on the death of the producing cell."

In these investigations the process was excellently demonstrated at the time of the very early stages of rhizomorph penetration into the Persian walnut root. As described before, the formation of this deep-staining material, which may now be called wound gum, was one of the first indications that the invading rhizomorph was acting below the cork layer. The browning of the cell wall and the formation of the wound gum appear to occur almost simultaneously; however, the wall sometimes browns without the formation of the gum. If the browning of the wall is indicative of the death of the cell and a normal-staining wall indicative of a healthy cell, then it is necessary to assume that the formation of the wound gum occurs with the death of the cell or shortly after. This would not support the view that it is a secretion of the living cell. If, on the other hand, the wall shows signs of browning before the protoplasm is dead, it may possibly mean that the wound gum is a secretion of the living cell. The former view seems rather more probable and is given some support in the myrobalan where at times there occurs a narrow zone of cells with yellowed walls between the normal tissue and the wound-gum area, thus indicating some disturbance, possibly death, ahead of the wound gum.

STAINING REACTIONS BEFORE COMPLETE DESTRUCTION

In the Persian walnut and myrobalan as in the potato, the narrow zone of tissue immediately surrounding the rhizomorph, when in the last stages before complete destruction, assumes a very deep red color with Flemming's triple stain and a rather dark brilliant green with Pianeze's. The colors, no doubt, represent the complete chemical change which occurs in the tissue as decay and distintegration proceed.

BROWNING OF THE WOODY CYLINDER

When an examination is made of the attacked woody tissues of the above hosts, the differences between the pear and the susceptible hosts are not at all striking. A common feature macroscopically observable in all the woody hosts, when *Armillaria* has formed a deep lesion and invaded the tissue below the cambium, is the browning of the woody cylinder, more noticeable on the side where the fungus is active, but sometimes penetrating deep into the cylinder in the vicinity of the lesion and extending up and down the wood a short distance. It affects the wood much deeper than any of the branching hyphae appear to penetrate. The browning is due to the occurrence in the vessels of the wood of a gummy-appearing material varying in color from a light-yellowish brown to a rather dark-reddish brown. It fills only a part of the wood vessels, the color of the wood becoming darker as more vessels are involved. It is believed that this is a different form of wound gum from that occurring within the cell. It may be a secretion into the vessels from these affected cells or a product from their walls. At first it is a light yellow and has much the same color as the cell wall in the region where formed, but it darkens with age. It does not ordinarily possess the granular appearance so noticeable in the red wound gum inside the cells of the myrobalan root. No destruction of the tracheal wall was observed at this stage of infection in any of the hosts examined.

FORMATION OF CORK

Cork formation around the infected area is not entirely limited to resistant hosts but was observed in all hosts examined, with the exception of the potato. Its occurrence is not constant in either susceptible or resistant roots, although when a lesion is formed and fails to spread a cork layer is often subsequently developed.

As first observed, a few cells in the parenchyma of the cortex several cells distant from the rhizomorph divide. A phellogen soon surrounds the affected area, and as this becomes active cork is formed. Since the stains employed do not clearly differentiate a suberin wall, it was impossible to be certain when the developing cells of this area first showed a suberin reaction. Freehand sections from many of these hosts stained with Sudan III took the typical deep-orange stain for suberin, when only a few layers were produced by the phellogen. The reaction may occur much earlier, but the proof is lacking. In a susceptible walnut this layer was never observed to attain a thickness of more than 3 or 4 cells, when surrounding an active rhizomorph. At about this stage it showed signs of disorganization, and further division of cells ceased. It is unknown whether or not suberin was deposited in these walls at this time.

It can be seen in the pear in plates 6, *A*; 7, *A* and *B*; and 8, *A*; and in the black walnut in plate 10, *A* and *B*, that cork forms and walls off the disorganized tissue around the rhizomorph. Plate 7, *A*, is an enlargement in the cork area. Although not illustrated, small lesions were found in the pear where no formation of cork had taken place and from appearances the rhizomorph was no longer active. In such cases, the dead, browned tissue may stop at as definite a line as if a cork layer were present. The dead cell is brown, but the one next to it may be perfectly normal in appearance. Instances of this nature lead one to question whether the cork is primarily a mechanical barrier preventing the spread of the fungus or merely forms after some other factor has checked the growth of the fungus. The latter seems more probable.

GUM CAVITIES

A discussion of the effect of *Armillaria mellea* upon deciduous fruit trees would not be complete without further mention of the gum cavities which occur in connection with this disease. So far as the author has been able to determine, no mention of this phenomenon has been made by anyone describing the effect of this disease on fruit trees. Of the many hosts examined it has never been found on any except those belonging to the genus *Prunus*. (The roots of *Citrus* have not been examined by the author.) Hartig (*16*), however, probably refers to a somewhat similar phenomenon in pine roots when he describes the abnormal resin and turpentine canals occurring in the region of the cambium. He claims that resinous materials flow into this region from the medullary rays, causing large abnormal canals in the "wood ring formed during the year of sickness."

In species of *Prunus* it has been a common observation that large quantities of gummy material exude from the base of many fruit trees when attacked by *Armillaria*. It often infiltrates into the surrounding soil where it dries and hardens to form a stony mass.

The formation of gum in species of *Prunus* has been investigated by many authors. A thorough discussion of its formation would be beyond the scope of this study. Butler (*9*), working with *Prunus* and *Citrus*, found its formation induced by "all manner of traumatisms" and that many chemical agents as well as various fungi were effective in producing it. He considers that the gum is due to the "hydrolysis of the walls of the embryonic wood cells", the action proceeding centripetally from the secondary lamella and finally reducing the whole cell to a mass of gum. Goldsworthy[3] credited bacteria with causing much of the gummosis in *Prunus*. It is thus seen that the production of gum may be due to a variety of causes, with the probability that organisms play an important role. There is no proof that the gum pockets here described are due entirely to the action of *Armillaria*. Bacteria might be present in conjunction with the fungus and produce the effect, but since other fungi cause gumming and since the phenomenon occurs almost constantly in connection with *Armillaria*, it is believed that the action is due to *Armillaria* alone.

Plate 11 represents typical gum cavities as observed in a prune tree attacked by *Armillaria*. The cavities most frequently occur in the layer of young wood cells just inside the cambium but are not limited to this region. At times they extend out into the phloem, apparently destroying the cambium. They are rarely confined to the phloem. If the root is small and completely girdled by the fungus, cavities may extend in a row entirely around the circumference of the root. Otherwise they extend as far as the fungus is operative. Occasionally two rows of cavities may form, one outside the other and rather close together. The medullary rays are seldom involved except as pressure may alter them. That pressure is developed in these cavities is evident from the manner in which the cells are compressed around the edges of the cavity; this suggests that while the tissue was young, gum was forced up from below into the area, and the cells were crowded apart and flattened tangentially around the gum column. At times very little solvent effect upon the cell walls is observable, but ordinarily some of the original cells are lost, probably contributing to the gum mass. Cavities are often found in which the cells surrounding them have inflated and extend into the cavity like tyloses into wood vessels. To explain this, it might be assumed that the pressure once developed later diminishes, allowing the still living cells around the sides to grow and expand into the cavity.

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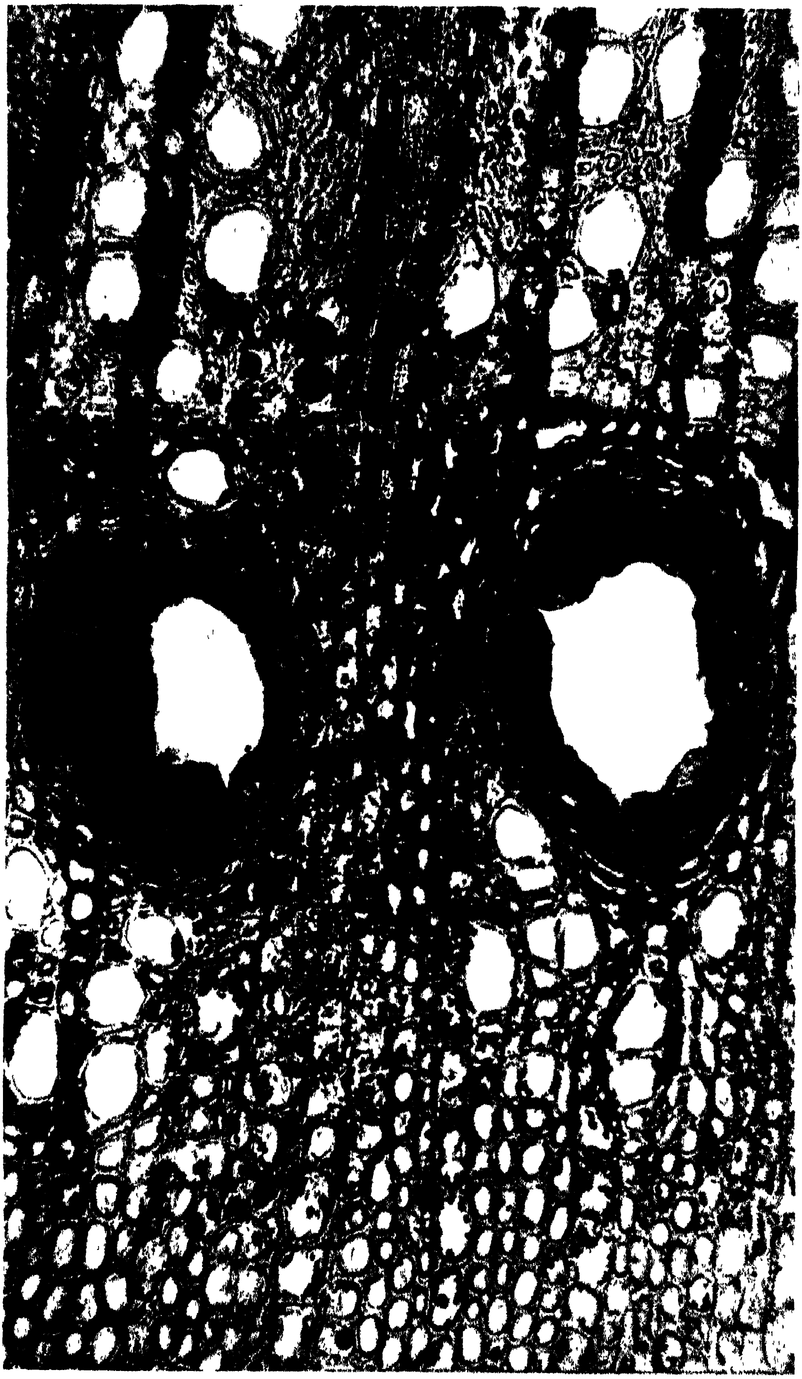
[3] GOLDSWORTHY, M. C. GUMMOSIS IN THE GENUS PRUNUS. (Doctorate thesis, Univ. Calif.)



Prune roots; gum cavities in young wood. × 263.

An examination of a longitudinal section of the affected tree trunk, including an area beginning with the healthy normal tissue above and extending down to the active rhizomorphs, would show somewhat the following: At the base of the section the rhizomorph is active in the cortex and cambium, sending out from its surface radiating hyphae which enter the outer layers of the wood as well as the cortex, act on and destroy it, and in so doing produce the gummy material, or produce substances which act on the young woody cells above, hydrolyzing their walls into this material, much as described by Hartig (*16*) in the case of the pines when turpentine or resinous material was produced. The gummy substances appear to follow more or less the vessels of the wood above the extending rhizomorph tip. The latter follows this gummy material that fills the cavities, destroying it or forcing it out as the rhizomorph proceeds upward. Many of the cells in this region are destroyed, indicating that the products of metabolism of the fungus are probably operative in destroying the young wood cells, similar to the chemical action described by Butler (*9*). During this destructive process the mass of gum is under pressure. Some of it is forced to the outside through the now disorganized cortex, and some is forced up into the young tissue around the cambial region, producing what has been observed before in cross section. As the fungus rhizomorph advances, this tissue is soon destroyed and the whole process gradually moves upward. This appears to be the most logical explanation, from the sectioning of many roots. The greatest distance which these cavities may extend up above the rhizomorph has received no critical attention, but they were observed to occur at a distance of 1 inch above the rhizomorph in a peach root.

GROWTH OF ARMILLARIA ON EXPRESSED SAP OF VARIOUS TREE ROOTS

In view of the results obtained in the histological and cytological investigations, indicating that resistance is a factor not concerned with any structural or morphological character of the host, a study was made of the fungus growth on the expressed sap of several tree roots, including both susceptible and resistant ones. The behavior of the fungus in attacking the resistant pear suggests that there may be some substance in the host which inhibits the growth of the parasite. If such an inhibiting substance is present in the cell sap and is of a stable nature not easily oxidizable or destroyed, it might be possible to test resistance by growing the fungus upon the expressed sap of the host.

A study of this nature was undertaken with the roots of several trees. The bark only was used, since the factor for resistance, if such be present, must be located in that portion of the root as well as in the wood. Roots to be tested were dug and protected from loss of water until such time as the sap could be expressed, when they were thoroughly washed and the bark peeled off and run through a meat grinder. The sap was then expressed from this ground material in a hydraulic plant press, using a pressure of 350 kilos per square centimeter. If any quantity of solid material was pressed out, the sap was centrifuged. As a means of sterilization the sap was filtered through a candle. In the first experiment a Chamberland-Pasteur filter was used, but in subsequent work a Berkefeld V was employed and with it a modification of the aseptic filter apparatus as described by Smith (*37*). The filtered sap was tubed in 3 to 5 cc lots, and in experiments after the first was allowed to stand for 3 or 4 days when two streaks were made from each tube to determine if contamination had occurred. This precaution was probably unnecessary as very few instances of contamination were ever found. The sap was then inoculated with *Armillaria* usually with the brown surface crust which forms when the fungus is grown on prune-agar slants. Care was necessary that the colony remain afloat because the production of internal rhizomorphs from a submerged colony was never observed. When growth starts at the surface the rhizomorphs develop after varying periods of time and grow down into the medium.

In the first experiment the roots of oak, *Quercus agrifolia;* fig, *Ficus carica* var. *sylvestris;* peach, *Prunus persica;* and apricot, *Prunus armeniaca* were employed. The oak and fig are fairly resistant, whereas the peach and apricot are quite susceptible. In this experiment the ground material was frozen before expressing, a procedure not used in later work. Some of the tubes in each lot were heated to stop enzymatic action. The data are given in table 1. Unfiltered sap was also inoculated, but in most instances it failed to support growth. Because of the probability of contamination little weight could be placed on the results, and they are therefore not reported.

TABLE 1.—*Growth of Armillaria mellea on the expressed sap of oak, fig, peach, and apricot roots*

[Sap expressed Mar 23, 1926]

Root	Sap treatment	Tubes	Tubes showing growth	Remarks
		Number	*Number*	
Oak	Filtered	5	4	Growth slow.
Do	Filtered and heated [a]	3	3	Do.
Fig	Filtered	5	5	Growth good
Do.	Filtered and heated [a]	4	4	Do
Peach	Filtered	5	0	
Do.	Filtered and heated [a]	4	0	
Apricot	Filtered	5	4	Growth less vigorous than on fig.
Do	Filtered and heated [a]	4	1	Do

[a] Placed in boiling water for 15 minutes. The contents of the tube reached 97° C

The most striking feature of these results is the fact that the fig and oak roots, which are decidedly resistant to *Armillaria*, showed very little inhibiting effect on fungus growth in the expressed sap, while the peach sap prevented growth entirely, yet the peach is one of the most susceptible of hosts. In all lots the heating of the sap almost to the boiling point had little significant effect upon the growth of the fungus. In the peach the growth-inhibiting substance is evidently thermostable.

Although the foregoing results gave little reason for believing that expressed sap might be a means of testing resistance, it was decided to make further trials, and in so doing to select a susceptible and a resistant species belonging to the same genus, in order that their dissimilarities might be less. The northern California black walnut, *Juglans hindsii* (resistant), and the Persian walnut, *J. regia* (susceptible), offered such a combination. The studies were carried on over a period of more than a year to determine if any seasonal change in the tree may affect the growth of the fungus on the expressed sap. A 3-year-old northern California black walnut (designated no. 1) growing in an "*Armillaria* spot" in an orchard was used in the experiment. Roots were removed from it at various intervals during a period of 17 months. Northern California black walnuts no. 2 and no. 3 were composite lots of roots of seedling trees grown in large pots for 2 years. The bark from the roots of several trees was composited and the sap expressed.

Persian walnut no. 1 was a chance seedling 4 or 5 years old. Persian walnut no. 2 was an old seedling planted as a border tree along the highway several years ago. Portions of the sap expressed on October 1, 1926, of both black walnut no. 1 and Persian walnut no. 2 were sterilized by adding a few drops of chloroform, shaking for a few minutes and evaporating off the chloroform by bubbling sterile air through it. This acted as a partial check against the possible removal in filtering of substances which might be essential as growth-inhibiting agents. Filtered, chloroformed saps were used as checks on the method. The chloroform treatment was found to have no influence on the growth of the fungus different from that obtained when the filter alone was used. It was therefore not used in later work. The data are presented in table 2. Growth failed on the sap from black walnut no. 1 in all trials except the first and the last. In the first, growth was good, but in the last trial only 2 out of 5 tubes supported growth and then only poorly. It is not believed that these differences are due to seasonal changes in the tree, for on July 20, 1926, the fungus growth on the sap was good but failed entirely on the same date 1 year later. That this factor of growth inhibition in the expressed sap is not constant in all black walnut trees is evident when the data relating to the black walnut seedlings no. 2 and no. 3 are compared with those of black walnut no. 1. In the former, growth was good, even better than on the supposedly susceptible Persian walnut, while in the latter it was almost a failure. Growth always occurred on the sap of the Persian walnut root, although not exceptionally good at all times. That the inhibiting factor in black walnut no. 1 is thermostable is evident from the results obtained with the sap expressed on July 20, 1927, which was heated in boiling water for a period of 15 minutes.

Table 2.—*Growth of Armillaria mellea on the expressed sap of northern California black and Persian walnut roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Black walnut no. 1.	July 20, 1926	Filtered	5	5	Growth fair.
Do.	Oct. 1, 1926	do	8	0	
Do.	do	Centrifuged, sterilized with chloroform	2	0	
Do.	do	Filtered, treated with chloroform	3	0	
Do.	Apr. 6, 1927	Filtered	8	0	[a]
Do.	July 20, 1927	do	11	0	
		Filtered and heated	5	0	
Do.	Dec. 22, 1927	Filtered	5	2	Growth poor.
Black walnut seedlings no. 2.	June 3, 1927	do	19	18	Growth very good.
		Filtered through Seitz filter	7	7	Do.
Black walnut seedlings no. 3.	June 2, 1927	Filtered	9	9	Growth good, but less than no. 2.
Persian walnut no. 1.	July 20, 1926	do	5	5	Growth poor.
Persian walnut no. 2.	Oct. 1, 1926	do	9	9	Growth fair.
		Centrifuged, sterilized with chloroform	3	3	Do.
		Filtered, treated with chloroform.	3	3	Do.
Do.	Mar. 26, 1927	Filtered	6	5	Growth good

[a] One month after first inoculation failed, one half of the tubes were heated in boiling water for 15 minutes and all reinoculated. Fungus again failed to grow in any tube.

Table 3 presents the results of culturing on the diluted sap of black walnut no. 1, using both prune decoction, which supports the growth of *Armillaria* very well, and distilled water as the diluting substances. Certain amounts of sap when added to prune medium are evidently invigorating to the growth of this fungus, and even when mixed in the high proportion of 1 part of sap to 1 of decoction growth is better than on the prune decoction alone, yet the expressed sap alone fails to support the growth of the fungus. When water is used as the diluting substance the results show that growth takes place and is best when the sap is diluted 1 to 1.

Table 3.—*Effect of dilution of the expressed sap from roots of resistant black walnut no. 1 on growth of Armillaria mellea*

Date expressed	Dilution no.	Volumes of—			Tubes	Growth
		Sap	Diluting substance			
			Quantity	Material		
		Cc	*Cc*		*Number*	
July 20, 1927	1	0.0	5.0	Prune decoction	5	Good, but not extremely vigorous.
	2	0.1	5.0	do	5	More vigorous than in dilution no. 1.
	3	1.0	4.0	do	5	Most vigorous of all lots with prune decoction.
	4	2.5	2.5	do	5	Vigorous, about like dilution no. 2.
	5	4.0	1.0	do	5	In 2 tubes only, and this poor.
	6	5.0	0.0	do	11	None.
Dec. 22, 1927	1	1	4	Water	5	Weak; similar to that in weak prune decoction.
	2	2	2	do	5	Most vigorous of water-dilution series.
	3	2	1	do	5	Slightly less than in dilution no. 2.
	4	3	0	do	5	In 2 tubes only, and this poor.

The results given in table 1 indicated that the expressed sap of the peach was inhibitive to the growth of the fungus. This was further tested with roots from the same tree, with roots from two other trees, and with a composite sample taken from a half dozen peach nursery trees. The results are given in table 4. In only two tubes did the fungus start and grow. Reinoculating after a period of 2 months showed that the growth-inhibiting substance was still present. Not only was growth inhibited but the inoculum was actually killed, for when removed and placed on prune agar, it failed to grow.

Table 4 also presents data regarding growth on the expressed sap of the roots of a composite sample of French pear seedlings. Growth was vigorous and rapid and differed little from that on the expressed sap of the susceptible Japanese pear (*Pyrus serotina*). On the sap expressed from myrobalan seedlings growth was positive but less vigorous than on the pear.

TABLE 4.—*Growth of Armillaria mellea on the expressed sap of peach, French pear, Japanese pear, and myrobalan roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Peach no 1	Feb 1, 1927	Filtered	8	1	Inoculum died in others.
	Apr. 1, 1927	Reinoculated	7	0	
Peach no. 2	Feb. 1, 1927	Filtered	5	0	Inoculum died.
	Apr 1, 1927	Reinoculated	4	0	
Peach (nursery trees)	Mar. 26, 1927	Filtered	6	1	
French pear seedlings	Apr. 26, 1927	do	9	9	Vigorous and rapid growth.
Japanese pear	Apr. 25, 1927	do	9	8	Do.
Myrobalan seedlings	May 5, 1927	do	14	11	Not as rapid growth as in pear

It is evident from these data that growth of the fungus on expressed sap of the bark of tree roots is not indicative of the susceptibility of the root to *Armillaria*, nor is the failure of growth indicative of resistance. The only instance of positive correlation was with black walnut no. 1; this varied and was not constant.

DISCUSSION

The modes of infection employed by root parasites have received little attention from investigators. A method of entry appearing to resemble most closely that of *Armillaria* was described by Peltier and Samson (*33*) in the case of *Ozonium omnivorum*. They state that by mechanical force hyphal wedges from the fungus strands on the root surface push in between the cork cells and finally engulf them in the fungus mass. The cells soon collapse but are not destroyed. Penetration occurs most commonly at a lenticel but may be directly through the cork. Another instance of root entry was described by Conant (*11*) who believed that the hyphae of *Thielavia basicola* in very young tobacco roots may mass together at times, and so weaken the suberized wall by enzymatic action that they are able to " surge " through. This, however, is not the usual method of entry. No single hyphae of this fungus were ever observed to penetrate a suberized wall.

It is stated by Appel (*1*) that species of *Phytophthora* and *Fusarium* penetrate a thin cork layer but not a thick one. Lutman (*27*), thought that the thickness of the skin may be partly responsible for resistance to potato scab. It might be argued in these cases that mechanical pressure is involved, otherwise the thickness of the cork would be relatively unimportant. Tisdale (*38*) in a study of flax wilt states that *Fusarium lini*, the cause of the disease, can penetrate the epidermis of the young roots; but when wound cork is developed around an infection inside the root, the fungus does not penetrate through it. He thinks it possible, however, that some reaction of the host protoplasm may weaken the fungus at the same time. Fawcett (*13*) states that *Pythiacystis citrophthora* is able to invade citrus roots through uninjured cork layer, but only if abundant moisture is present with favorable soil temperatures over a sufficiently long period of time.

From the fragmentary evidence presented in the literature it might be concluded that root parasites do not commonly enter through the uninjured and healthy cork layer, but occasionally, when this does occur, they may enter either by means of mechanical force or chemical dissolution of the cork wall. *Armillaria* seems to present a more definite case of entry through the thick cork layer of a comparatively old root than any other fungus hitherto reported. According to the results of this investigation, the usual method by which *Armillaria mellea* gains entry into a root is by penetration of a rhizomorph branch directly through the sound cork layer into the tissue below. Of the numerous infections examined, no instance of definite entry through the ruptured tissue around a newly formed branch root where it emerges from the parent root, as suggested by Zeller (*46*), and by Rayner (*35*), was observed. Occasionally entrance is gained through a lenticel, and in such instances the method is similar to that through the cork layer. Under no circumstances was there evidence of a splitting of the cork cells at their middle lamellae. The rhizomorph branch enters as a unit, apparently employing both mechanical and chemical means in its penetration of the root periderm. It appears to be almost unique in its method of forcing through the suberized walls of the cork layer, as a single unit, the comparatively great bulk contained in a rhizomorph branch.

Kusano (*25*) and Day (*12*) concluded that this fungus had the power of destroying suberized tissue when the rhizomorph branch penetrated, and the evidence presented in this study gives further support to the same view. If the cork is thus actually dissolved it would seem necessary that some enzyme be associated with the process. A search of the literature failed to reveal that any specific enzyme produced by micro-organisms and capable of attacking suberin has ever been demonstrated. On this point Waksman (*42*) states "so far as our present information is concerned, cork and cutinized lamellae are not acted upon to any extent by micro-organisms." The action upon the cortex cells by *Armillaria* is not extensive and might fall within Wakman's "not acted upon to any extent," but where a group of cells is actually dissolved, as occurs in this case, the process could hardly be placed in that class. Since the normal action by micro-organisms is enzymatic, it would seem that some enzyme may be involved in this case.

The evidence presented establishes beyond much doubt that *Armillaria mellea* can readily enter the sound, healthy roots or tubers of both susceptible and resistant plants. The apparent ease with which the fungus enters through the cork layer of all the hosts investigated makes it seem doubtful whether resistance to this fungus in any plant can be due entirely to the prevention of entrance. It would seem more logical that the second act of the fungus, the establishment of parasitic growth in the host, is the feature that decides whether this fungus is or is not to become a parasite. If such is the case, wounds would not play such an important part in this disease as other authors have assumed. They may, however, have a secondary effect in facilitating the establishment of the fungus by affording it saprophytic nourishment.

Conant (*11*) in his study of *Thielavia* root rot of tobacco decided that fungal invasion stimulated phellogen development in advance of the fungus and was of the opinion that the layer of cork was effective in walling off the fungus. Butler (*8*) discusses the defensive action of what he terms reactionary cork or that developed as the result of damage by invading parasitic fungi. He cites shot hole and pear and apple scab as examples of diseases where reactionary cork forms and prevents further spread of the fungus. He states that it often happens in these diseases if the fungus is growing vigorously that the plant is unable to form a continuous corky layer and consequently unable to prevent penetration into the tissue below at all points. A new layer of cork may then form and the process be repeated several times. He apparently considers, in the cases mentioned, that the formation of the reactionary cork is primary in function in the walling-off of the parasite and is not in any way secondary. In *Armillaria* root rot a phellogen is often produced far in advance of the penetrating rhizomorph and is especially noticeable in the resistant roots. It might be assumed that it is walling off the fungus and preventing its spread. The rhizomorph readily penetrates cork tissue, however, and it would therefore be expected that little good would be accomplished by the secondary periderm. That this is the case is proved by the instances found in which the rhizomorph had apparently penetrated directly through the second cork layer. It would thus appear that in the pear the formation of the wound cork is not a factor responsible for the resistance exhibited by this host. Other instances noted, in which the action of the rhizomorph had apparently ceased without the development of cork around it, lends more weight to the belief that cork formation is not a factor of resistance.

Studies on the growth of the fungus on the expressed sap show no significant correlation between inhibition of growth and resistance of the host. Of the resistant species, only in the root of a single black walnut was growth inhibited on the expressed sap, and even in this root, when the sap was added to prune decoction upon which the fungus ordinarily grows well, when not too concentrated, it actually produced more vigorous growth of the fungus. It, however, remains an unproved possibility in this particular instance that the osmotic pressure of the expressed sap of this black walnut may be sufficient to inhibit fungus growth. In case this factor is responible it is not a constant one for the expressed sap of all resistant roots Hawkins (*19*) is of the opinion that parasitic fungi will grow on solutions with a much higher osmotic pressure than the expressed sap of their hosts. The failure of *Armillaria* to grow on the expressed sap from peach roots may possibly be due to the products developed in the enzymic destruction of the glucoside amygdalin. In tubes containing expressed sap from peach roots the odor of hydrocyanic acid was always very distinct.

Several authors including Butler (*7*), Vavilov (*41*), Howitt (*23*), and Walker (*43*) have given reviews of the literature on disease resistance in plants, pointing out the principal factors responsible for resistance. In the present work on *Armillaria mellea* the histological evidence does not demonstrate that structural differences of the hosts are concerned. The primary and secondary cork layer proved to be an ineffective barrier. In fact, there seems to be no proof that resistance is of a mechanical or morphological nature; or as Vavilov (*41*) classifies resistance, it is not a case of "mechanical or passive immunity." If one follows Vavilov it must then fall in his other class, "physiological or active immunity." Certain factors are obviously unimportant in this class.

Positive or negative chemotropism in the sense suggested by Massee (*28*) would not explain the entrance into the resistant host with subsequent inhibition of the parasitic action. If chemotropism was a factor initiating penetration, it would not be expected to hinder growth after invasion had once occurred.

The acidity of the cell sap as a factor concerning resistance is a much debated question, but it is doubtful if it would be at all concerned in *Armillaria* resistance. Wolpert (*45*) has shown that an acidity corresponding to a pH value of from 2.0 to 2.9, varying with the media used, was necessary to inhibit the growth of *Armillaria* in artificial culture. It is doubtful if the acidity of the cell sap would ever approach this figure.

The fungus penetrates through the cortex of the resistant pear root and into the wood below the cambium, at times killing and destroying some wood. The fungus and its parasitic action finally come to a standstill, cork forms in the cortex walling off injured tissue. The cambium around the edges of the lesion produces new wood and cortex tissue, which in time heals over the wound. These facts lead one to believe that there is some antagonistic factor concerned with the tissue of the root which finally overcomes the parasitic action of the fungus and prevents its further spread. Furthermore, this factor appears to be limited to the active, healthy tissue of the root as evidenced by trials in which 2- to 3-inch root cuttings of the pear were placed in test tubes containing a few cubic centimeters of water and inoculated on the upper cut end. The fungus grew down the cambium quite readily. Such roots can hardly be considered in an active, healthy state; neither can they be considered as dead. From these trials one can at least conclude that there is nothing in morbid tissue of a pear root which inhibits growth. This observation, together with the fact that the fungus grows very readily on the expressed pear sap, gives strong evidence that resistance in the pear must be concerned with the healthy growing tissue.

Klotz (*24*) suggests that resistance in *Citrus* to *Pythiacystis citrophthora* may be of the nature of a paralyzing or inhibiting effect upon the enzymes produced by the fungus by some substance present in the plant. If this is an explanation of resistance to *Armillaria*, it must be assumed that the paralyzing factor in the host tissue is not expressible with the cell sap or is in some way changed and rendered inactive by this procedure. Otherwise it could hardly be expected that *Armillaria* would grow so vigorously on the expressed sap, unless the enzymes of the fungus concerned with parasitism are different from those concerned with saprophytism.

While the manner in which the plant is attacked and overcomes the effect of the parasite might be thought of as something similar to antibody production in the animal system, there are no data to support such a belief.

From the evidence presented in this investigation one is led to believe that resistance is due to some antagonistic factor contained in living, healthy plant parts, which cannot be expressed with the cell sap, and is not present to any degree in morbid tissue.

SUMMARY

This paper presents the results of an investigation to determine the mode of entrance and subsequent development of *Armillaria mellea* in various susceptible and resistant roots and tubers. Observations on the growth of the fungus on expressed sap of various susceptible and resistant hosts are included. There exists a possibility that such studies might throw some light upon the nature of resistance.

Invasion of the root is accomplished by the penetration of a branch of the parent rhizomorph directly through the sound, healthy periderm of the host. The method is similar in the different hosts investigated, with no apparent difference between susceptible and resistant ones.

The branch penetrates as a unit and was never observed to send out single hyphae into the host ahead of it.

Penetration is partly by mechanical and partly by chemical means. There appears to be some destruction of the suberized walls as if they were acted upon by a suberin-dissolving enzyme.

Death of the cells always precedes the further advance of the rhizomorph into the tissue. In susceptible hosts the killing usually extends further away from the rhizomorph than in the resistant ones.

In susceptible roots, after entry has once been gained, the rhizomorphs grow rapidly and cause general destruction of the host tissue.

In resistant roots the fungus readily gains entrance but is unable to establish itself and ordinarily destroys but little of the affected root. The wounds thus formed soon cork out or heal over.

Wound gum was observed in the border of the lesions in some hosts. It was most noticeable in the walnut and myrobalan.

A secondary cork layer often forms in resistant hosts, walling off the wound made by the invasion of the fungus. Its significance

as a factor pertaining to resistance is doubtful, since the fungus readily breaks through such layers.

Gum cavities, which are of almost constant occurrence in species of *Prunus* affected by this fungus, are described and discussed.

The fungus grows well on the expressed sap of certain roots and not at all or only very poorly on others, but there seems to be little correlation between the inhibition of growth in this manner and the resistance of the living host.

Structural or morphological differences of the host probably exert little influence on resistance.

Resistance to *Armillaria mellea* appears to be of the nature of an antagonistic influence exerted upon the fungus by the host only when the latter is in an active, healthy state.

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DETERMINATION OF HARDINESS IN ALFALFA VARIETIES BY THEIR ENZYMATIC RESPONSES[1]

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INTRODUCTION

In addition to the outright winter-killing of various overwintering crops, cold injury causes a greater reduction in yield than is generally realized. A reduction of from 46 to 82 percent has been found in the growth of the first crop of alfalfa after root injury produced by low temperatures (*22*).[2] Often such crops as cotton or rice are injured by temperatures above freezing (*27*), and these add an important group to the long list of economic plants directly affected by low temperatures. Considerable progress has been made at the Nebraska and other stations in the development of an accurate method of artificial freezing for differentiating varieties according to their cold resistance. This method is a tool that may be used to advantage in both differential and improvement work and in further studies of the fundamental nature of winter hardiness. The solution of the last-named problem is the ultimate objective of winter-hardiness investigations.

This paper reports the results of a study of the relation of varietal hardiness and the hardening-off condition in alfalfa to enzymatic activity, with particular reference to the diastatic enzymes. In this investigation the diastatic enzymes were studied only through the sugar-forming amylase, the starch-liquefying enzyme receiving no direct consideration. While the chief object of the study was to establish, if possible, a method for determining varietal hardiness by means of differences in diastatic activity, it was also hoped to obtain information on a phase of the winter-hardiness problem that has heretofore received little attention.

One of the reasons for investigating this phase of the problem is the consistency with which certain investigators have correlated sugar content with winter hardiness in various plants. Since the time of Lidforss (*15*) attention has been drawn to the fact that there is often an increase of sugars during the hardening process. Recently Åkerman (*1*) has established varietal differences in wheat by means of their sugar content. Newton and Brown (*19*) have indicated the importance of sugar concentration in protecting the protein complex of the plant. Thus the fact has been established that sugar content plays an important part in the problem of winter hardiness. Assuming the importance of sugar concentration, the question immediately arises, What changes occur within the plant to promote the increase of sugars? Since many plants have a high diastatic power, it is

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[2] Reference is made by number (italic) to Literature Cited, p 239.

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logical to attribute changes in the sugar content to the diastatic activities within the plant. To diastase and other enzymes and to carbohydrates might be attributed the carbohydrate equilibrium which exists within the cell at any given time. This may be true whether the increase in sugar during the hardening period is due to conversion of starch to sugar, as is apparently the case in many plants, or whether the increase in sugar is not at the expense of starch, as Tumanov (*29*) suggests is the case with wheat and related crops.

While the sugar concentration is a primary reason for the study of the diastatic enzymes, the importance of enzymes in general as factors in the life processes of plants is sufficient to warrant their investigation in connection with such a problem as winter hardiness.

Although the writer has found nothing in the literature bearing directly on the relation of diastatic enzymes to winter hardiness, a considerable amount of work has been done on various enzymes in relation to overwintering. This is particularly true of catalase and the enzymes connected with respiratory activity. An extended review of the literature on various enzymes as related to winter hardiness is outside the scope of this paper. A very brief review, however, indicates that at the lower temperatures lower rates of respiration for the more hardy than for the nonhardy varieties have been found by Govorov (*10*), Martin (*16*), Samoylenko (*26*), and Newton and Anderson (*17*) in wheat and rye, and by De Long (*4*) in apple twigs.

Newton and Brown (*18*) in a single series of experiments found a sharp index of the relative hardiness of wheat varieties in their catalase activity, the more hardy having the higher activity.

Kling (*14*) reports differentiating cold resistance of winter cereals by their protein ferments.

Literature bearing on the study of the relation of diastatic enzymes to the winter-hardiness problem will be considered in the discussion of the results.

MATERIAL AND METHODS

A considerable amount of preliminary work, extending over a period of 2 years, was done before the procedure now in use was adopted. Since very few of the preliminary experiments are reported, only a detailed account of the present procedure is given.

OBTAINING THE SAMPLE

For the main test reported in this paper, four varieties,[3] namely, Turkistan (F.C. 15754), Grimm (F.C. 15713), Nebraska Common, and Arizona Common (F.C. 15837), were planted May 13, 1931, in nursery rows. When samples were desired in the fall the plants were dug to a depth of approximately 6 inches and taken to the greenhouse, where the roots were trimmed and quickly but thoroughly washed. Each sample consisted of about 5 inches of the root below the crown, the crown tissue and tops being removed. The crown tissue was removed in order to secure a more uniform sample, some of the plants having a very large woody crown and others having much less. Immediately after the roots were cleaned and the surface moisture allowed to evaporate, the roots were weighed for the samples. A very small quantity is sufficient for an analysis, as little as 5 to 10 g being used in some instances. To insure a larger and therefore more representative population of plants, however, a sample consisting of 75 g of roots was used in each of the tests. A corresponding sample of approximately the same weight was taken for the dry-weight determinations.

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[3] For convenience the term "varieties" will be used throughout this paper, although it is not strictly from the botanical viewpoint.

After being weighed, the sample was ground in an ordinary, easily cleaned meat chopper. Three hundred cubic centimeters of distilled water saturated with toluol was then added to the 75 g of ground root material and the whole thoroughly shaken and allowed to extract at room temperature overnight, usually 20 hours. The samples for dry-weight determinations were placed for 24 hours in an electric oven maintained at 100° to 105° C.

DETERMINATION OF ORIGINAL AND PROTECTED DIASTATIC ACTIVITY

At the end of 20 hours' extraction the material was filtered through a no. 12 Whatman folded filter paper and the saccharifying power of the filtrate determined. Various studies concerning time of extraction and filtering as compared with nonfiltering indicated that within reasonably wide ranges no great differences in results were obtained. Considerable care, however, was exercised in using a uniform method of procedure throughout an experiment.

The term "original activity" is used to designate the diastatic activity as determined directly from the root extract. The term "protected activity" is used to designate the diastatic activity as determined after exposing the extract to a temperature of 70° C. for 10 minutes.

The term "protected activity" refers to the apparent protection of the enzyme by the medium in which it is found, enabling it to withstand high temperatures. For example, a determination by this method showed that the diastatic activity of saliva was greater than that of an equal quantity of alfalfa-root extract, but that when heated to 70° C. the diastatic activity of saliva was reduced practically to zero, whereas in some instances in the fall the diastatic activity of alfalfa-root extract, as will be shown, is reduced only 50 percent.

To determine the original diastatic activity, 2 cc of the filtrate (which is also called the extract) was added to 25 cc of 2-percent potato-starch solution prepared by the Lintner method, which had been brought to the digestion temperature of 30° C. by means of a constant-temperature water bath. The enzymes were allowed to act upon the starch for a period of 40 minutes. As will be shown later, it is important to keep this period the same in all tests.

The amount of reducing sugar in the starch-enzyme mixture at the end of the digestion period was determined by the picric-acid method suggested by Blish and reported by Blish, Sandstedt, and Platenius (*2*). Briefly, the method used consisted in transferring 1 cc from the digestion to a tube containing 2 cc of saturated aqueous solution of picric acid and 1 cc of saturated aqueous solution of sodium carbonate. This was shaken and placed in a boiling-water bath for 30 minutes. After the tubes had been removed from the water bath the contents were made up to 10 cc by adding distilled water, and were ready for comparison in a colorimeter against a known standard made up from a standard maltose solution in the same manner. For further discussion of the picric acid method the reader is referred to Willaman and Davison (*32*).

In addition to the sample taken from the extract for the activity determination, a similar sample was taken for the blank. This was heated for 10 minutes in a boiling-water bath and then added to 25 cc of the 2-percent starch solution. Since this solution retained no diastatic activity, a determination of the amount of reducing sugar gave a value which when subtracted from the amount of sugar found after digestion would show the amount of reducing sugar that could be attributed to the diastatic activity.

An additional check was made by determining the amount of sugar in the extract and in the starch solution, separately, by the picric acid method. When added together in the proper proportions, these results usually gave approximately the value found for the blank by the other method. It was found that heating the extract in the boiling-water bath for 10 minutes did not interfere with the amount of reducing sugars to such an extent as to make the results unreliable.

To determine the protected activity, a 2-cc aliquot from the extract was placed in test tubes especially measured for uniformity in thickness of wall, and these were placed in a water bath kept at exactly 70° C. for 10 minutes. After the tubes had been cooled 1 minute in tap water, 25 cc of starch solution was added immediately to each tube and the whole shaken and placed in the constant-temperature bath at 30°. Digestion continued for 40 minutes, as in the original-activity determination, and in all other respects the same procedure was followed in the determination of the protected activity as in the determination of the original activity.

Although the digestion was allowed to proceed only 40 minutes, for the sake of clarity all results are calculated on the hour basis and in terms of dry matter, that is, in activity per gram of dry matter per hour.

Throughout the procedure great care was taken in pipetting. Automatic pipettes were used where possible, and in other instances care was taken not to allow saliva to contaminate the tubes. Checks made on the use of traps in pipetting showed that no measurable contamination was involved in the present method, in which traps were not used.

In all the work duplicate or triplicate activity determinations were made for each extract. Usually good checks were obtained between the duplicates, the error not exceeding 5 percent.

INFLUENCE OF TIME AND TEMPERATURE

As stated by Gortner (*9*) and others, in determining enzymatic activity it is absolutely necessary to consider time as a factor. This is no doubt particularly true in the experiments under consideration, because a limited amount of starch solution was used and the concentration of the end product increased as the action progressed.

In figure 1 the rate of increase in sugar concentration and the diastatic activity per gram per hour has been plotted against time. Two cubic centimeters of alfalfa-root extract was placed in 25 cc of 2-percent starch solution; 1-cc samples were withdrawn at intervals, and the activity was determined on these in the usual manner. The curve indicating the maltose produced, in milligrams per gram per hour, shows how exceedingly rapid the activity was during the first few minutes. If the action had continued at the same rate thereafter the extract from 1 g of alfalfa root would have produced the equivalent of 35 g of maltose from starch in 1 hour. Actually, however, presumably because of the limited amount of starch available, there was produced at the end of 1 hour only 2.44 g of sugar. The reduction in activity with advancing time is shown by the slope of the curve in figure 1.

The actual increase in reducing-sugar accumulation in the starch-enzyme mixture is also shown in figure 1. The increase in sugar was very rapid for the first 10 minutes, but the accumulation was less rapid, though consistent, from that point up to 2 hours, when it reached 13.2 mg maltose per cubic centimeter of digestion. That the mixture had not reached equilibrium even after 2 hours is indicated by the fact that at the end of 26½ hours the concentration of maltose had increased to 17.7 mg per cubic centimeter of digestion.

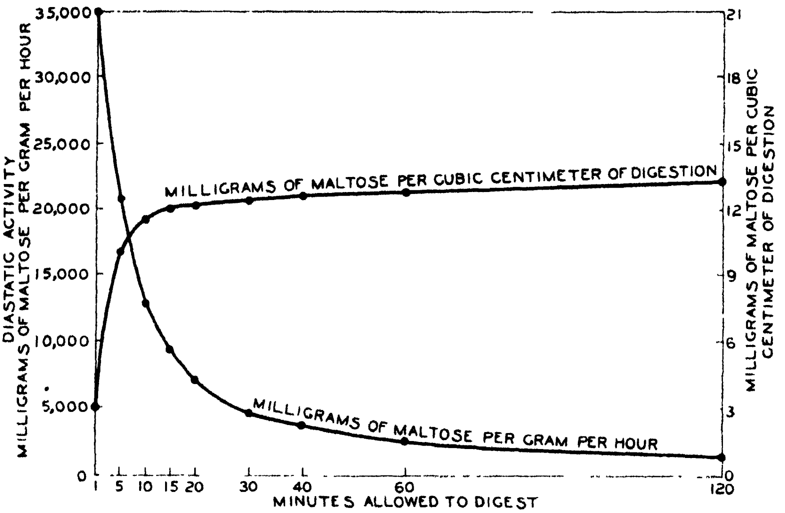

FIGURE 1.—Influence of period of digestion on diastatic activity of alfalfa-root extract, measured in milligrams of maltose per cubic centimeter of digestion and milligrams of maltose per gram of root (dry matter) per hour.

The length of time allowed for digestion for the activity determination is subject to variation depending upon the object of the test. To determine the point of starch-sugar equilibrium a long period must be used; to determine the activity a short period must be used when there is a limited amount of starch. In the tests reported in this paper, a somewhat intermediate period of 40 minutes was chosen with the expectation that it would serve both to give an indication of activity and also the ability of each variety to swing the starch-sugar equilibrium in the sugar direction.

A similar period of 40 minutes for digestion was chosen for determining the protected activity. Throughout the tests the 40-minute period for digestion was used unless otherwise specified, even though in most of the tabular data the activity is calculated on the per hour basis.

Figure 2 shows the results of exposing alfalfa-root extract to various high temperatures previous to determining diastatic activity. This test was made on an extract of Nebraska Common alfalfa, the sample being obtained during the summer, which would account for its low activity at 70° C.

Five 2-cc aliquots, in duplicate, were heated in the water bath for 10 minutes, at five different temperatures. The first set was heated at 58° C., the second at 62°, and so on at 4° intervals up to 74°. As a check, the original activity was determined without any heating. As will be seen from figure 2, the heating at 58° did not change the activity of the extract to any extent.

Heating at 62° C., however, instead of decreasing, slightly increased the activity of the sample. Additional tests showed that heating at a temperature just below that at which injury occurs apparently stimulates activity. At 66° the enzyme was injured slightly by heating and showed a reduced activity, at 70° the reduction was much greater, and at 74° there was very little activity. This reduction in activity is not constant for different extracts, as it depends on the condition of the extract. For example, an extract from hardened plants in the late fall does not show so great a reduction at 70° C.

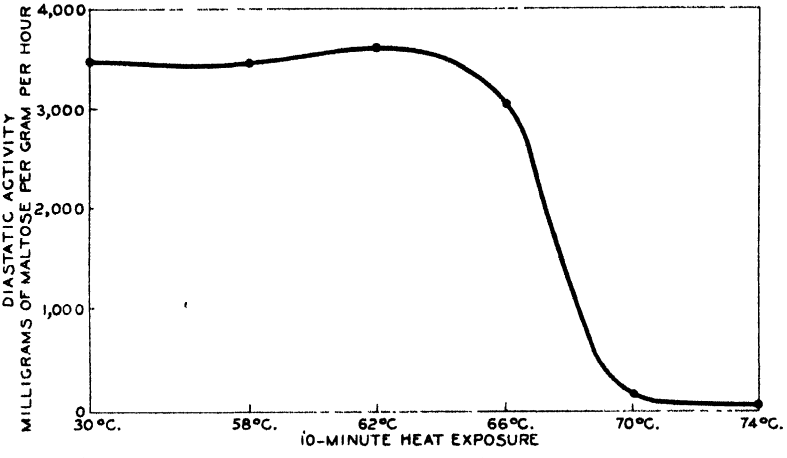

FIGURE 2.—Effect of exposing extract from alfalfa roots to various high temperatures for 10 minutes previous to determining diastatic activity; the activity determinations were made at 30° C.

Instead of the rate being 121 mg of maltose per gram per hour, as in this case, it has been found to be from 280 to 1,443 mg of maltose per hour. (See table 5.) A uniform procedure of heating to 70° was adopted throughout, as this temperature brings out best the differences in the hardened condition without being too severe for unhardened material.

RELATION OF DIASTATIC ACTIVITY OF ALFALFA TO VARIETAL AND SEASONAL DIFFERENCES

ORIGINAL DIASTATIC ACTIVITY OF HARDENED AND UNHARDENED ALFALFA TOPS AND ROOTS AT VARIOUS TEMPERATURES

One of the objects of the enzymatic study was to determine whether the hardy varieties had a greater diastatic activity than the nonhardy varieties, particularly in the hardened state, and whether there was any change in the enzymatic activity during the hardening process.

In one of the first experiments the tops of the hardy variety Turkistan and the tops of Utah Common, a relatively nonhardy variety, were compared in both the hardened and the unhardened condition. In both cases the unhardened material exhibited greater activity than the hardened material. The hardened Turkistan had a slightly greater activity than the hardened Utah tops; this difference, however, was not very great.

A second test was conducted on 2-month-old plants of the hardy variety Grimm, and a nonhardy variety of Arizona Common. In this experiment both tops and roots were used separately. It was found that the roots of both varieties had approximately five times as great diastatic power, per gram of dry weight, as the tops. Again it was found, particularly in the tops, that the nonhardy variety had the greater activity. In these experiments, as in later ones, results consistently showed greater activity in tissues having the most rapid growth, but did not serve to differentiate hardy from nonhardy varieties.

The next experiment was the first to indicate a deviation from the previous experiments, and is particularly important in that it formed the basis for future work on heating tests. In this test the material (alfalfa tops) was dried in an oven at 65°–67° C. for 30 hours. It was then ground and extracted, and the enzymatic activity was determined. Contrary to former results, the hardened material showed greater activity than the unhardened. This reaction, although not understood at the time, served to stimulate further research along this line and formed the starting point for more extensive work on the protected diastatic activity. Another interesting feature of this experiment was the determination of the enzymatic activity at lower temperatures. It was thought that perhaps the hardy varieties would show greater activity at lower temperatures (near 0°), corresponding to their ability to harden. This, however, did not prove to be the case, since near 0° as well as at 30° Arizona Common had a slightly higher activity than Grimm.

ORIGINAL AND PROTECTED DIASTATIC ACTIVITY OF ALFALFA VARIETIES

WINTER OF 1930-31

Early in the fall of 1930, after the previous year's work had indicated that the activity of the enzyme after heating had some relation to winter-hardiness, certain changes were made in the method to insure greater accuracy in the determinations. It was at this time that the present methods were adopted which, as previously stated, involved using fresh, macerated material for extraction, filtering the extract, and taking aliquots from this extract for the determinations. In addition, a constant-temperature water bath was substituted for the hot-air oven, and a uniform time of exposure for the protected-activity determination was adopted. It was found that the small quantity of extract used, when placed in a 20-mm test tube and immersed in the water bath, would reach approximately the temperature of the water bath in 2½ minutes. In order to allow sufficient time for the heated material to come to the temperature of the water bath and to remain at that temperature for a short time, a uniform period of 10 minutes was adopted.

For the first test in which this method was used, 1-year-old alfalfa roots of the Arizona Common and Turkistan varieties were dug from the field December 19, 1930. The roots were trimmed just below the crown so that no bud tissue was included in the sample. The roots were then washed, weighed, and ground. The results (table 1) show very little difference in the original activity but a striking difference in activity after heating, the activity of Turkistan being much less reduced by heat than that of Arizona Common. The original activity of both samples was high, producing more than 4,600 mg of maltose per gram per hour, and there was not a great difference between the two varieties. If the activity of Turkistan is taken as 100 percent, the activity of Arizona Common is 92 percent. On the other hand, if the activity of the heated extract of Turkistan is taken as 100 percent, that of the heated extract of Arizona Common is only 22 percent.

TABLE 1.—*Original and protected diastatic activity of roots of Turkistan and Arizona Common*

Variety	Diastatic activity (maltose per gram per hour)	
	Original	Protected [a]
	Milligrams	*Milligrams*
Turkistan [b]	5,003	2,291
Arizona Common [b]	4,612	494
Turkistan [c]	5,022	75
Arizona Common [c]	2,643	23

[a] Activity of extract after being heated 10 minutes at 69° C.
[b] Determinations on extracts were made immediately after preparation.
[c] Determinations on extracts were made Jan. 17, 1931, 28 days after preparation.

The extract from the Turkistan and Arizona Common varieties was kept in the laboratory at room temperature until January 17, 1931, when a duplicate set of activity determinations was made. The results given in the last half of table 1 indicate very strongly that the hardy variety maintains its original activity better than the nonhardy variety. In other words, the protective power which enables the enzyme from the hardy variety to withstand heat to a greater extent than that from the nonhardy variety also enables it to withstand the effects of being in a mixture with water for a considerable length of time. It will also be noted that changes have occurred which make the enzymes unable to withstand heat as well as when the samples were fresh. Thus, when the Turkistan extract that had been held in the laboratory was heated to 69° C. for 10 minutes on January 17, it retained an activity of only 75 mg, as compared with 2,291 mg for the freshly prepared extract. The Turkistan variety, however, still maintained a superiority over the Arizona Common variety. Hydrogen-ion determinations by the colorimetric method showed clearly that the extract had become more acid.

A second experiment was undertaken which included the four varieties Turkistan, Grimm, Kansas Common, and Arizona Common. The samples were obtained December 22 from the field, when there was about 4 inches of frost in the ground. The roots were treated as in the previous experiment, the extract being heated to 69° C. The results are given in table 2. Here again, while there are no great differences between the original activity of any of the varieties, the activity after they had been heated sharply and distinctly separated

Grimm from Turkistan, Kansas Common from Grimm, and Arizona Common from Kansas Common, and in the same order as in field and artificial-freezing tests (*21, 23*).

TABLE 2.—*Original and protected diastatic activity of 4 alfalfa varieties taken from field plots Dec. 22, 1930*

Variety	Diastatic activity (maltose per gram per hour)	
	Original	Protected[a]
	Milligrams	*Milligrams*
Turkistan	4, 494	2, 161
Grimm	4, 855	1, 789
Kansas Common	4, 734	1, 536
Arizona Common	4, 197	1, 066

[a] Activity of extract after being heated 10 minutes at 69° C.

At least three other varieties, namely, Ladak, Nebraska Common, and California Common, were tested by the protected-activity method and found to give results which placed them in their respective winter-hardy positions.

Several attempts were made to use germinating seedlings in the hardened and unhardened state to determine their relative hardiness by the enzymatic method. Seedlings 3 to 15 days old, hardened for 4 to 10 days, served to bring out reliable differences between varieties. However, since the differences were neither so large nor so consistent as those obtained from field plants, the latter were employed in all tests made during the present investigation.

WINTER OF 1931–32

Following the method outlined above, the four alfalfa varieties Turkistan, Grimm, Nebraska Common, and Arizona Common were planted in rows on May 13, 1931. They were clipped in August, when they were in the full-bloom stage. Moderate growth took place after the clipping, and on September 24, when the first samples were taken, from 6 to 10 inches of growth had occurred, depending upon the variety. The roots had developed to a relatively large size, since the plants were sown in rows, and only 15 to 18 roots were required to make up the 75-g sample.

Sampling was continued throughout the winter and early spring at 2-week intervals. Table 3 gives the dates of sampling, the mean minimum temperature for the 2 weeks previous to sampling, and the height of the plants at the time of sampling. Additional data are given on the condition of the soil, and snow covering, and on general plant conditions at time of sampling.

The high minimum temperatures of early fall made possible the late growth of the Nebraska Common and Arizona Common varieties. Grimm and Turkistan also continued growth, but were checked by shortening day length, particularly Turkistan, much more than were the common alfalfas. The temperatures were unusually high for so late in the fall, and this, together with the continued vigorous growth of the common varieties, should be borne in mind in interpreting the results obtained.

TABLE 3.—*Meteorological data and soil and growth conditions at time of sampling*

Date of sampling [a]	Mean minimum temperature for 2 weeks previous to sampling	Height of plants				Remarks
		Turkistan	Grimm	Nebraska Common	Arizona Common	
1931	°*F.*	*Inches*	*Inches*	*Inches*	*Inches*	
Sept. 24	66.0	6	7	9	10	Very warm
Oct. 8	56.0	10	12	14	15	Growth continued, particularly Nebraska Common and Arizona Common.
Oct. 21	47.1	10	12	15	16	Growth slowing up
Nov. 4	43.1	6	7	14	14	First frost of year Nov. 1, minimum temperature 28°, tops frosted
Nov. 17	40.2	----	----	----	----	Growth stopped by frost
Dec. 7	27.7	----	----	----	----	Dormant.
Dec. 21	28.5	----	----	----	----	Do
1932						
Jan. 4	35.6	½-1	1	1-1½	1-1½	8-inch snow, ground not frozen, slight growth during warm spell the week previous to sampling, cold immediately preceding sampling
Jan. 18	13.0	----	----	----	----	8- to 10-inch snow; ground not frozen.
Feb. 1	12.2	----	----	----	----	5- to 10-inch snow, frost 7 inches in ground
Feb. 15	17.2	----	----	----	----	No snow, frost averaged 6 inches deep
Feb. 29	27.4	----	----	----	----	No frost in ground, signs of bud growth
Mar. 14	14.9	----	----	----	----	2-inch snow, frost 10 inches in ground
Mar. 28	29.6	1½	1½	1½	1	New growth begun
Apr. 11	41.5	7½	7½	7½	6½	Arizona Common showed slight winter injury none of the others injured
Apr. 25	46.4	16	17	17	17	Vigorous growth

[a] The original and protected diastatic activities for alfalfas sampled at these dates are given in tables 4 and 5

Table 4 gives the original diastatic activity of the four varieties for each date sampled during the 8-month period from September 1931 to April 1932, inclusive. The period is divided into two parts, the first ending January 4. This division was made for several reasons, but chiefly because there seemed to be a change in the response of the plants at about this date, as will be seen later. The same division is followed throughout the discussion of the other factors under consideration. For brevity the first 8 dates will be referred to as the "fall" and the last 8 as the "spring", although the winter months are included.

Possibly the most important objective was the determination of varietal and seasonal differences. From the standpoint of varietal differences, it is apparent that no variety is consistently higher or lower in diastatic activity than the others, either in the fall or in the spring. In the fall Turkistan had a slightly higher average, and Grimm ranked second. In the spring Turkistan ranked first, and Arizona Common second.

The seasonal difference was somewhat more striking, the average of all the fall determinations for all varieties being 3,250 mg of maltose per gram per hour, while in the spring it was 3,975 mg. Moreover, there were no exceptions among the varieties, all showing higher activity in the spring than in the fall. The greatest activity is found on the last date, April 25, when the plants were 16 to 17 inches high and growing vigorously.

Table 4.—*Original diastatic activity in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per gram (dry weight) per hour				
	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept 24	3,298	3,163	3,424	3,145	3,258
Oct 8	3,465	3,615	3,598	3,996	3,669
Oct 21	3,000	2,986	2,857	3,145	2,997
Nov 4	3,017	2,815	2,664	3,122	2,905
Nov 17	3,187	3,177	2,898	3,075	3,084
Dec 7	3,274	3,159	3,185	3,138	3,189
Dec 21	3,465	3,496	3,330	3,028	3,330
1932					
Jan 4	3,648	3,767	3,499	3,357	3,568
Average	3,294	3,272	3,182	3,251	3,250
1932					
Jan 18	3,850	3,772	3,667	3,690	3,745
Feb 1	3,473	3,245	3,124	3,364	3,302
Feb 15	3,980	3,968	4,027	4,053	4,007
Feb 29	4,330	3,875	3,912	3,824	3,985
Mar 14	4,108	3,768	3,697	3,813	3,847
Mar 28	4,548	4,404	4,002	4,124	4,270
Apr 11	4,359	3,969	4,151	4,410	4,222
Apr 25	4,706	4,164	4,272	4,555	4,424
Average	4,169	3,896	3,857	3,979	3,975

It should be pointed out here, however, although it will be dwelt upon in further detail later, that another and apparently important reason for the increased activity shown as late winter advances is due to the decreasing percentage dry weight of the roots. Since the data are on the dry-weight basis, in the case of high percentage dry weight in the roots the factor used to bring the results to milligrams per gram (dry weight) per hour would be relatively smaller than the factor used when the sample contained a much smaller dry weight.

Entirely different results were obtained in regard to the protected diastatic activity. In regard to varietal responses the differences among the varieties in the fall were very striking, as shown in table 5, where the average protected activity of triplicate samples of each variety for each date is given. These data are summarized in simpler form in table 6, where the varieties are ranked according to their protected diastatic activity.

During the fall period Turkistan ranked first on 7 of the 8 dates, and on the eighth date ranked second. Grimm varied somewhat more from second place, but maintained a fair difference over Nebraska Common in third place. Arizona Common was consistently low and ranked fourth 6 times out of 8, definitely taking last place. The average protected diastatic activity of Arizona Common was slightly higher than that of Nebraska Common, but this can be accounted for by the unusually high values for Arizona Common on October 21 and January 4. When the root reserves, which will be discussed later, are considered, it will be difficult to explain not why Arizona Common was so high but how, in spite of a fall development that particularly favored Arizona Common for root storage, the protected diastatic activity was so low as to indicate that this variety was the least winter-hardy of the four.

TABLE 5.—*Protected diastatic activity in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per gram (dry weight) per hour				
	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept. 24	414	332	272	248	317
Oct. 8	289	248	228	188	238
Oct. 21	1,186	586	744	1,031	887
Nov. 4	1,096	590	652	550	722
Nov 17	1,443	1,147	627	497	929
Dec. 7	1,239	739	368	335	670
Dec 21	939	317	622	280	540
1932					
Jan. 4	968	1,213	354	886	855
Average	947	647	483	502	645
1932					
Jan. 18	672	499	742	868	695
Feb. 1	1,148	587	377	447	640
Feb 15	506	500	795	331	533
Feb 29	361	278	326	245	303
Mar. 14	300	371	291	148	278
Mar 28	66	172	146	156	135
Apr 11	38	20	75	70	51
Apr 25	105	49	103	177	109
Average	400	310	357	305	343

TABLE 6.—*Rank, based on protected diastatic activity, of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common
1931					1932				
Sept. 24	1	2	3	4	Jan. 4	2	1	4	3
Oct. 8	1	2	3	4	Jan. 18	3	4	2	1
Oct. 21	1	4	3	2	Feb 1	1	2	4	3
Nov. 4	1	3	2	4	Feb 15	2	3	1	4
Nov. 17	1	2	3	4	Feb. 29	1	3	2	4
Dec. 7	1	2	3	4	Mar. 14	2	1	3	4
Dec. 21	1	3	2	4	Mar. 28	4	1	3	2
					Apr. 11	3	4	1	2
					Apr 25	2	4	3	1

It is interesting to note that the protected-activity data for the spring do not serve to bring out the varietal differences in their correct order. While Turkistan has a slightly higher average, the rank of any variety, as shown in table 6, is not sufficiently consistent to establish the superiority of any one variety. For the period from February 15 to March 14, however, Arizona Common ranked fourth. During the latter part of this period the most severe temperatures of the winter were recorded, and frost penetrated 10 inches into the ground, the greatest depth recorded during the winter. It is significant that the only winter injury found the following spring was in Arizona Common. The injury, which was slight, doubtless occurred during this period.

The trend with respect to the seasonal difference is also of especial interest. In the early fall the average protected diastatic activity for the four varieties was only 238 to 317. On October 21 it rose very rapidly to over 800 and maintained a high value for 6 weeks. For the next 4 weeks the protection of the enzymes gradually decreased, but was again high on January 4. It is interesting to consider the mean minimum temperatures for the 2-week periods preceding sampling. For the fall period a decrease in protection occurred when the mean minimum temperature was above 50° or below 32° F. On the other hand, the large increases in protected activity occurred when the preceding mean minimum temperatures were between 32° and 50°. Thus it appears that hardening occurs at the latter temperatures. The same relation holds true for the late winter period, with the additional fact that where the minimum temperatures are quite low, as between 12° and 14°, the change in protection, although downward, is not so rapid as when the minimum temperatures are just below freezing, as at 27° and 29°. It is probable that this can be explained on the basis of more rapid respiration at the higher temperatures. The fact that rapid changes occurred within apparently dormant plants indicates the labile condition of living plant tissue.

The gradual but consistent decrease in protection to the diastase against heat as the season advances from winter to spring is very striking and is logically correlated with the reduced food reserves indicative of the early spring "critical period" mentioned by Steinmetz (*28*), Peltier and Tysdal (*22*), Saltikovsky (*25*), and others.

The very lowest point in protected diastatic activity, namely 51, was reached after growth had started vigorously and the plants had attained a height of about 7 inches. When the samples were taken on April 25 the new growth was about 17 inches in height, and by that time there was a slight increase in the protected activity. Further studies are being pursued throughout the summer to determine the reaction of the diastase in relation to growth.

RELATION OF ENZYME ACTIVITY OF ALFALFA TO SUGAR IN EXTRACT AND TO DRY MATTER IN ROOTS

As previously stated, the reducing sugar (calculated as maltose) in the extract and the dry matter of the roots was determined. The values found for the quantity of reducing sugar per cubic centimeter of extract are given in table 7, and the percentages of dry matter in the roots in table 8. Although the data in table 7 are not represented as being a correct analytical procedure for determining root reserves, the values found should bear a rather definite relation to the total carbohydrate reserves in the root, inasmuch as the root mass is ground and the diastatic enzymes are allowed to act for a period of 20 to 24 hours in the carbohydrate material of the root. That this method should give an accurate indication of the carbohydrate root reserves is not essential for present purposes, except that it would be of interest as showing the general trend of reserves, which is strikingly illustrated in the data from early fall to late spring. It is also interesting to note that the least hardy variety, Arizona Common, has the highest average amount of sugar in the extract.

TABLE 7.—*Maltose per cubic centimeter of extract from roots of 4 alfalfa varieties sampled at 2-week intervals from October 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Oct. 21	11.60	9.31	8.57	9.80	9.82
Nov. 4	5.99	6.46	7.53	6.40	6.60
Nov. 17	7.13	7.87	9.96	10.86	8.96
Dec. 7	5.11	5.28	6.52	8.42	6.33
Dec. 21	4.85	5.57	5.79	8.98	6.30
1932					
Jan. 4	4.24	5.62	5.50	8.57	5.98
Average	6.49	6.69	7.31	8.84	7.33
1932					
Jan. 18	5.39	5.75	6.35	9.69	6.80
Feb. 1	4.62	4.57	4.52	5.50	4.80
Feb. 15	3.12	3.34	3.23	5.50	3.80
Feb. 29	3.49	4.72	4.75	6.62	4.90
Mar. 14	2.48	3.29	3.46	5.37	3.65
Mar. 28	2.82	3.25	3.10	4.27	3.36
Apr. 11	2.62	2.59	2.49	3.53	2.81
Apr. 25	2.99	2.53	2.54	2.65	2.68
Average	3.44	3.76	3.81	5.39	4.10

TABLE 8.—*Dry matter in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sept. 24	32.8	32.7	30.9	32.7	32.3
Oct. 8	32.5	32.5	31.6	30.3	31.7
Oct. 21	36.7	36.1	35.9	33.9	35.7
Nov. 4	35.9	38.0	39.9	34.3	37.0
Nov. 17	35.5	36.0	38.7	36.0	36.6
Dec. 7	33.1	33.5	34.3	34.5	33.9
Dec. 21	30.5	30.0	31.6	32.7	31.2
1932					
Jan. 4	30.9	29.3	32.3	32.4	31.2
Average	33.5	33.5	34.4	33.4	33.7
1932					
Jan. 18	28.8	29.5	29.6	30.0	29.5
Feb. 1	28.5	29.9	29.6	28.5	29.1
Feb. 15	27.9	28.0	28.5	27.9	28.1
Feb. 29	26.5	28.9	27.9	28.9	28.1
Mar. 14	24.7	26.7	26.7	25.3	25.9
Mar. 28	23.1	24.1	26.3	25.5	24.8
Apr. 11	20.5	20.7	21.5	20.9	20.9
Apr. 25	20.5	20.0	20.1	19.5	20.0
Average	25.1	26.0	26.3	25.8	25.8

For the purpose of the present study it is sufficient to know that the data given, are, within the error of determination, the actual amounts of sugar present in the extract when the determinations were made of the original and protected diastatic activity. The data may therefore be compared directly with the activity determinations presented in previous tables. Similarly, the dry-matter percentages given in table 8 may be compared directly with the activity determinations. Comparisons between the various sets of data were made by means of the correlation coefficient, values of which are as follows:

Correlations between—	*Coefficient*
Original activity and protected activity	−0.5259
Original activity and percentage dry matter	−.9057
Protected activity and percentage dry matter	.5624
Protected activity and milligrams of maltose per cubic centimeter of extract	.5167
Milligrams of maltose per cubic centimeter of extract and percentage of dry matter	.8280

The partial correlation between protected activity and percentage of dry matter, where milligrams of maltose per cubic centimeter of extract was held constant, gave a value of 0.2802; while the partial correlation between protected activity and milligrams of maltose per cubic centimeter of extract, where percentage of dry matter was held constant, gave a value of 0.1106.

The multiple correlation between protected activity and both percentage of dry matter and milligrams of maltose per cubic centimeter of extract was 0.5697.

Judged by the level of significance as given by Fisher (*8*) all the simple correlations are significant. It is interesting to note the high negative correlation between original activity and percentage of dry matter, which confirms the previous statement that the lower the dry matter the higher the factor for changing activity into terms of per gram per hour and consequently the higher the activity. The relatively high positive correlations between protected activity and dry-matter content or milligrams of maltose per cubic centimeter of extract are of especial importance in showing a relationship between these factors. This relationship is to be expected on the basis of the protective action of sugar. The correlation might very easily be considerably higher if each variety could be analyzed separately, because the fact that Arizona Common is highest in amount of sugar in the extract yet one of the lowest in protected enzymatic activity doubtless tends to reduce the correlation coefficient. Possibly the fact that Arizona Common and Nebraska Common had a much greater growth in the fall than the other two varieties may account for the difference in the amount of reducing sugar in the extract.

As would be supposed, there is a very high positive correlation between milligrams of maltose per cubic centimeter of root extract and percentage of dry matter of the root. This close relationship apparently greatly reduces the partial correlation coefficients when either factor is eliminated. When both are considered in relation to protected activity in the multiple correlation, the resulting coefficient is somewhat higher than either of the simple correlation coefficients.

RELATION OF STARCH-SUGAR EQUILIBRIUM IN DIASTASE DIGESTION TO SEASONAL DIFFERENCES

From figure 1, where the activity of the enzyme in 2-percent soluble starch is shown, it may be seen that the rate of sugar accumulation is very slow during the diastase digestion after about 30 minutes; and, although it has not reached equilibrium, as shown by the fact that sugar continues to increase for at least 20 hours, it may be said to be approaching equilibrium. A tabulation of the quantity of sugar in the diastase digestion might show whether varieties differed in their ability to produce a higher concentration of sugar in the digestion, and whether the differences were due to the seasonal change from fall to spring. The data (table 9) are taken directly from the determinations made for the original activity, being the amount of sugar in milligrams per cubic centimeter produced by the enzymes in 2 cubic centimeters of the root extract in a 2-percent starch solution in 40 minutes.

TABLE 9.—*Sugar concentration produced in 40 minutes on 2-percent starch solution at 30° C. by diastase of root extract from 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per cubic centimeter of digestion of starch by diastase from—				Average
	Turkistan	Grimm	Nebraska Common	Arizona Common	
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept. 24	13.36	12.81	13.08	12.69	12.99
Oct. 8	13.92	14.52	14.04	14.93	14.35
Oct. 21	13.58	13.32	12.65	13.15	13.18
Nov. 4	13.36	13.21	13.11	13.21	13.22
Nov. 17	13.96	14.12	13.84	13.67	13.90
Dec. 7	13.35	13.05	13.49	13.36	13.31
Dec. 21	13.06	12.95	12.99	12.21	12.80
1932					
Jan. 4	13.93	13.64	13.94	13.43	13.74
Average	13.57	13.45	13.39	13.33	13.44
1932					
Jan. 18	13.69	13.72	13.40	13.67	13.62
Feb. 1	12.24	11.97	11.42	11.85	11.87
Feb. 15	13.66	13.73	14.17	13.95	13.88
Feb. 29	14.19	13.84	13.46	13.66	13.79
Mar. 14	12.51	12.41	12.17	11.93	12.26
Mar. 28	12.95	13.12	12.98	12.97	13.01
Apr. 11	11.05	10.11	11.00	11.40	10.89
Apr. 25	13.42	11.57	11.95	12.32	12.32
Average	12.96	12.56	12.57	12.72	12.70

The data for fall and spring are given separately, as in previous tables. The averages obtained show that there is a greater amount of sugar produced in a given length of time in the fall than in the spring. The difference, though not great, is consistent for all varieties. The average for all fall determinations is 13.44 mg of maltose per cubic centimeter of digestion; for spring determinations, 12.70 mg. Since this is to be expected if changes in the cell contents in the fall stimulate or cause a shift in the starch-sugar equilibrium, the results are considered significant. The varietal differences are neither so great nor so consistent. In the fall, however, the Turkistan variety had a slightly higher average diastatic power, producing 13.57 mg per cubic centimeter; Grimm was second, Nebraska Common third, and Arizona Common fourth. This order corresponds with the relative hardiness of the varieties. In the spring the differences did not follow the same consistent trend. It is suggested, therefore, that as hardening develops within the plant certain changes enable the diastatic enzymes to shift the starch-sugar equilibrium in the sugar direction. This theory does not necessarily conflict with the finding that Arizona Common had the highest average amount of sugar in the root extracted, since it is probable that this variety had a much higher total carbohydrate content in its roots when the samples were obtained.

RELATION OF PROTECTED DIASTATIC ACTIVITY TO COLD RESISTANCE

The ability of enzymes to resist heat or deleterious substances, such as salts of heavy metals, alcohol, etc., is influenced by the medium in which the enzyme is acting. This fact has been established by Euler and coworkers in Sweden (*6*), Willstätter and his coworkers in Germany (*33*, *34*), and others.

O'Sullivan and Tompson (*20*) found that to destroy invertase in solution required a temperature fully 25° C. higher when cane sugar was present than when it was absent.

Willstätter, Graser, and Kuhn (*33*) and Willstätter and Wassermann (*34*) show that while the activity of yeast saccharase may be but slightly affected by purification, its heat inactivation is greatly altered. The straight yeast extract showed no inactivation at 52° C., whereas a purified preparation was reduced 43 percent in its activity.

Haldane (*11, pp. 69–70*) states:

> However, purified enzymes are generally much more thermolabile than crude preparations, and the increase in lability often coincides with the disappearance of protein reactions. The critical temperature and temperature coefficients are certainly sometimes and possibly always, those of protective proteins or other colloids associated with the enzyme, and not properties of the enzyme itself.

Vernon (*30*) indicates that the presence of proteins and impurities acting as protective colloids or buffers serves to protect the enzyme against the inactivating effect of high temperatures. Various salts, such as phosphates and chlorides, may also serve as protective agents. It is suggested that they probably form heat-resisting compounds with the enzyme.

Hudson and Paine (*12*) show that fructose acts as a protective agent against destruction of invertase by acid, alkali, or hot water. They further state (*13*) that cane sugar exerts a protective action against alcohol, 6 percent of the sugar reducing the rate of destruction, in 50-percent alcohol, to 1 percent of its original value.

Rockwood (*24*) found that the addition of sufficient aspartic acid to make the concentration of the whole mixture 0.01 N increased the action of ptyalin 52 percent. In addition he found that the amino acid served to prevent decay of the enzyme. Glycine was also found to stimulate the action of ptyalin.

Fales and Nelson (*7*) found that at optimum pH of invertase action the salt effect of sodium chloride in the concentrations used approached zero, but if the pH was altered to either side of the optimum there was an increased inhibitory salt effect.

Various workers have found that potassium salts within certain concentrations increase the activity of diastatic enzymes. Waksman and Davison (*31*) place potassium salts near the top of the list as inorganic activators.

Englis and Lunt (*5*) found that the addition of potassium salt increased diastatic activity of plants grown in peat but not in sand. They suggest that perhaps the lowest potassium content was sufficient for optimum activity in sand.

In this connection it is interesting to note that Yasuda (*35*) has found that a deficiency of potassium inhibits the formation of sugar and the plants become less hardy. A high application of potassium under low temperature increases the sugar content of the plants, which become consequently hardier.

In general, therefore, it can be stated that the kind and amount of carbohydrate and of protein or protein derivatives, the colloid content, the salt content, and the amount of various other substances have a direct bearing on the activity and thermostability of the diastatic enzymes. In order to show the influence of a few of these factors on the protected activity of alfalfa-root diastase the following tests are reported.

INFLUENCE OF DEXTROSE, HYDROGEN-ION CONCENTRATION, AND AMINO ACIDS IN THE MEDIUM ON PROTECTED ACTIVITY OF ALFALFA-ROOT DIASTASE

To determine the effect of sugar protection on the diastase of alfalfa roots, solutions containing 10, 20, and 40 percent dextrose were made up, and 2 cc of each added to separate 2-cc aliquots of the root extract. In addition 2 cc of distilled water was added to another aliquot, and as a check another aliquot was used without the addition of any other substance. These were heated in duplicate in the hot-water bath at 70° C. for 10 minutes.

The results shown in figure 3 indicate that a very high degree of protection against heat resulted from the addition of dextrose. The concentration in the extract was 1.4 percent dextrose, and this when heated to 70° C. had a subsequent diastatic power of 1,640 mg, equaling 51 percent of the original activity, i. e., the activity of the unheated extract. When 2 cc of 10-percent dextrose was added, however, making the total concentration 5.7 percent dextrose, the protection increased to such an extent that the subsequent activity was 98 percent of the original. Similarly at a concentration of 10.7 percent the activity was 95 percent of the original, but at 20.7 percent the protective power was decreased so that the activity was only 58 percent.

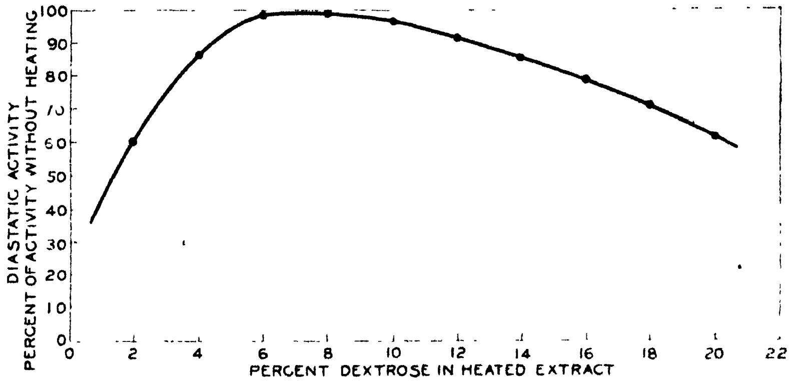

FIGURE 3.—Influence of dextrose in alfalfa-root extract on resistance of diastatic enzymes to heating at 70° C. for 10 minutes, activity without heating taken as 100.

These data indicate that optimum protection is afforded when the concentration in the heated sample contains from 6 to 10 percent dextrose. This corresponds very well to the findings of Newton and Brown (*19*), who state that a concentration of about 8 percent of either sucrose or dextrose gives optimum protection against protein coagulation in expressed juice of winter wheat.

An analogous test with the amino acid glycine showed that the addition of 2 cc of 1 N glycine to 2 cc of extract gave 63 percent more activity than that of the extract alone when heated to 70° C., and that 3 N glycine gave more than double the activity. In this test the activity of the extract alone was very low after the extract had been heated, being only 7.1 percent of the original activity.

The pH of the solution also had an important influence on the action of the enzymes and on their ability to withstand heat. McIlvaine's buffer mixture of disodium phosphate and citric acid, as outlined by Clark (*3, p. 241*), was used to give buffers of 2.2, 4.0, 6.0, and 8.0 pH. Five 75-g alfalfa-root samples were taken as usual, but in addition to the one in which the distilled water was used for extraction, 300 cc of the buffer at each of the above pH's was used for each of the other four samples.

The pH of the buffer and the pH of the mixture of root tissue and buffer as represented in the extract, together with the original and protected activity, are given in table 10.

It will be noted that the cell sap of the root itself forms a strong buffer and that the sodium phosphate-citric acid buffer does not remain at its original pH, but that the final pH as determined by the colorimetric method is much nearer the pH of the water extract than the pH of the buffer mixture.

The activity of the enzyme is zero at the low pH of 3.1, but at the next higher pH the original activity is almost as high as at the optimum pH of between 5.3 and 5.6. On the other hand the protected activity is very low at pH 5.2, reaching a maximum at pH 5.3 and gradually decreasing at 5.6 and 6.7. Thus the protected activity is more sensitive to the hydrogen-ion concentration than is the original activity, and the results again show the influence of a very slight change in pH on the reaction of the enzyme to heat.

CONCLUSIONS

The experiments reported herein show some of the factors which may actually increase or decrease the protected activity of the diastatic enzymes. The interesting feature is that many of the substances that are here shown to increase the resistance to heat of the diastatic enzymes of alfalfa-root extract are the very substances which various workers have suggested increase in the plant during the hardening process. If this is the case, and it seems certain that it is, the underlying principle of differentiating hardiness by the protected activity seems sound.

Table 10. *Influence of hydrogen-ion concentration on the original and protected diastatic activity of alfalfa-root extract*

pH of buffer	Actual pH as determined by colorimeter	Diastatic activity (maltose per cubic centimeter of digestion)	
		Original	Protected [a]
		Milligrams	*Milligrams*
2.2	3.1	0	0
4.0	5.2	3,139	4
Distilled water	5.3	3,246	78
6.0	5.6	3,334	54
8.0	6.7	3,163	11

[a] Activity of extract after being heated 10 minutes at 70° C.

To say, then, that any one factor such as sugar, colloids, etc., is responsible for the particular enzymatic response is to preclude the possibility of other important constituents being factors, all of which may have a profound bearing on the physicochemical complex. It is suggested, rather, that the ability of the enzyme to withstand heat is a test of the stability of the entire cell-constituent complex.

SUMMARY

This paper presents the results of a 3-year study of the diastatic activity of alfalfa roots and tops.

It was found that the diastatic activity of alfalfa tops is closely correlated with the rapidity of growth and not with the hardiness of the varieties, even in the fall under hardening conditions. The activity in the roots does not decrease, however, with dormancy. Alfalfa roots have about five times as much diastatic activity per gram of dry matter as alfalfa tops from the same plants. Early studies indicated the importance of determining the original activity (the diastatic activity of fresh root extract) and the protected activity (the diastatic activity after the extract has been subjected to a temperature of 70° C. for 10 minutes). The methods used and the small amount of equipment necessary for determining both the original and protected activity are given in detail.

These two determinations were made on the four alfalfa varieties Turkistan, Grimm, Nebraska Common, and Arizona Common at semimonthly intervals for a continuous period of 8 months, from September to April inclusive. It was found that the varieties did not differ widely and that there was no great seasonal difference in the original activity. It was found, however, that a higher concentration of sugar could be produced from a limited amount of starch by enzymes taken from plants in the fall than from those taken in the spring, and that during the fall a higher concentration could be produced from the hardy varieties than from the less hardy varieties.

The protected activity gave the greater and more consistent values with respect to both seasonal trends and varietal differences. A rapid increase of protected activity occurred in all varieties in the fall, reaching a maximum from October to late November and extending into January; afterward there was a gradual decrease, reaching a minimum in April, more than 2 weeks after growth started and when the new growth was 6 to 8 inches high. The protected activity increased more rapidly and maintained a higher value in some varieties than in others; the hardier the variety the higher was the protected activity. In general, the varietal differences between the four varieties Turkistan, Grimm, Nebraska Common, and Arizona Common were marked and consistent in the fall. After midwinter, however, the differences diminished, and toward spring no one variety showed a distinct superiority.

Supplemental experiments showed that the concentration of sugar in alfalfa-root extract influences the protected activity, as does also the concentration of amino acids. The hydrogen-ion concentration was also shown to influence the resulting protected activity. Since an increase in some of these substances occurs in the plant during hardening, and since these substances increase the resistance of the diastatic enzymes to heat, the relation between winter hardiness and protected diastatic activity is suggested. This relationship, together with the

experimental results obtained, forms the basis for suggesting this as a method for determining varietal resistance to cold as well as for further fundamental studies on the problem of winter hardiness and related phenomena.

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CUTTING YIELDS OF HOGS AN INDEX OF FATNESS[1]

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INTRODUCTION

Fatness is one of the major factors affecting the market value of hogs and hog carcasses. The preference of consumers for the leaner cuts of meat and the competition of vegetable oils have resulted in comparatively low prices for lard, fat cuts, and very fat hogs. Breeders and research workers are consequently endeavoring to select and establish types and strains of hogs that will produce the most desirable proportion of lean to fat. To do this they are conducting breeding trials, performance tests, registers of merit, and hog-carcass contests. A basic problem in this work is to determine as accurately as possible the comparative fatness of the carcasses produced.

Comparisons of fatness, on the market, are made by observing or judging the appearance of the carcass This method is neither accurate nor permanent enough for the definite comparisons needed by constructive breeders or research workers

The most accurate means of determining the fatness of a hog carcass is obviously a chemical analysis of the carcass. The time, expense, and quantity of meat involved in this method, however, often make it impracticable. Chemical analysis also entails the loss of the pork for other experiments. It would be desirable to have more simple measurements, if their relation to the fatness of the carcass could be established.

Three types of measurements seem particularly adapted for determining the actual fatness of a pork carcass. In the order of probable accuracy they are as follows: (1) The chemical analysis of some single, representative cut; (2) the weight of particularly fat or lean cuts in relation to the weight of the entire carcass; and (3) measurements of parts of the carcass, especially the fat portions, such as thickness of the fat on the ham, shoulder, and back.

The second type of measurement—that of estimating the fatness of an animal from the proportions of the fat or lean cuts in the carcass—was selected as the basis for the study reported in this paper. The existence of a relationship between these weights is indicated by the work of other investigators as well as by that of the writers. Moreover, the procedure was more practicable than an analysis of a selected cut and was thought to be more accurate than measurements of the fat layers of hogs of various weights. This paper also includes a

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This study was conducted as a part of the national project, cooperative meat investigations, formerly designated as study of the factors which influence the quality and palatability of meat

[2] Acknowledgment is made to Mrs E V. Steely, of the Animal Husbandry Division, who was largely responsible for assembling and correlating the data

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statistical analysis of the relationship between the comparative weights of selected lean and fat cuts and the fat content, as measured by the ether extract, in the entire carcass. These data, obtained from the records of 75 hog carcasses, were then applied to a much larger number to show how the relationship may be used in the study of the fatness of swine.

The specific problems undertaken were: (1) To determine the mathematical relationship between the percentage yields of selected cuts and actual fatness; (2) to determine the mathematical relationship between the ratio of fat to lean cuts, and the actual fatness of the carcass; (3) to establish a series of percentages for selected cuts that could be used to express the fatness or to estimate the fat content of the total edible portion of hog carcasses; and (4) to check the use of this series as an index for determining the fatness of groups of hogs and of individual hogs.

REVIEW OF LITERATURE

A relationship between the percentage of cold carcass weights represented by the respective cuts and the apparent fatness of hogs has been reported by a number of investigators, Bull and Longwell (*1*)[3], Scott (*8*), Schmidt, Vogel, and Zimmermann (*7*), Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*), and Mohler (*4*). All agree that, as hogs fatten, the proportionate weight of the carcasses represented by the fat cuts increases and that of the lean cuts decreases. These same trends can also be observed in the standard yields of packing-house test departments. Obviously those parts containing the higher proportion of bone and muscle, such as ham and loin, constitute a greater proportion of the thinner carcass. As the hog fattens, proportionately more weight is added in the fat cuts, such as belly, leaf, and back fat.

The relationship between the percentage yield of the cuts and the actual fatness, or ether extract, in the carcass was indicated by the reports from the Illinois station. Little or no difference in the percentage yield of fat cuts from hogs of various types was reported by Bull and Longwell (*1, pp. 451-453*). This apparent similarity in fatness was proved when Mitchell and Hamilton (*3, pp. 572-575*) analysed the same carcasses and found no significant difference in fat content.

A ratio between the weights of selected fat and lean cuts was found to vary consistently with the apparent fatness of hog carcasses, according to Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*), and Schmidt, Vogel, and Zimmermann (*7*). Changes in this ratio were also reported to be associated with corresponding changes in the actual fat content of the carcasses, as determined chemically by Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*). The fat content of the carcasses studied by these investigators ranged from 37.27 to 56.17 percent. This range compares favorably with the range of fat content of carcasses analyzed by Ellis.[4]

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[3] Reference is made by number (italic) to Literature Cited, p. 255.

[4] Unpublished data.

TABLE 1.—*Average live weight at slaughter of 5 groups*[a] *of hogs, classified by weight, breed, and sex*

ALL 598 HOGS

Breed	Heavy (250 pounds or more)				Medium (200-249 pounds)				Light (160-199 pounds)				Light-light (130-159 pounds)				Pig (less than 130 pounds)			
	Barrows		Gilts		Barrows		Gilts		Barrows		Gilts		Barrows		Gilts		Barrows		Gilts	
	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter
	No	*Lb*	*No*	*Lb*	*No*	*Lb*	*No*	*Lb*	*No*	*Lb*	*No.*	*Lb*	*No*	*Lb*	*No*	*Lb*	*No*	*Lb*	*No*	*Lb.*
Chester White	16	294	9	300	42	219	30	217	29	182	42	182	8	148	5	142	5	113	1	84
Poland China	19	296	17	295	32	215	26	219	24	184	24	186	3	146	3	147	6	113	2	113
Duroc-Jersey	5	286	2	325	21	218	10	218	10	176	17	185	3	147	5	146	2	79	3	98
Tamworth	16	267	8	275	42	220	31	222	22	184	25	182	5	143	9	147	4	114	3	103
Crossbreds and others					2	232	4	213	3	189	3	180								
Total or average	56	286	36	293	139	218	101	219	88	183	111	183	19	146	22	146	17	109	9	101

THE 75 HOGS ANALYZED CHEMICALLY

Breed	Heavy Barrows Hogs	Heavy Barrows Lb	Heavy Gilts Hogs	Heavy Gilts Lb	Medium Barrows Hogs	Medium Barrows Lb	Medium Gilts Hogs	Medium Gilts Lb	Light Barrows Hogs	Light Barrows Lb	Light Gilts Hogs	Light Gilts Lb	Light-light Barrows Hogs	Light-light Barrows Lb	Light-light Gilts Hogs	Light-light Gilts Lb	Pig Barrows Hogs	Pig Barrows Lb	Pig Gilts Hogs	Pig Gilts Lb
Chester White	5	302	1	295	4	221	4	225	3	193	9	182					5	113		
Poland China	3	282	2	337	3	202	4	214	1	193	6	194					1	109	1	111
Duroc-Jersey			1	330	5	223	2	208	2	177	3	191							1	99
Tamworth					1	201														
Crossbreds and others							1	200	3	189	2	177					2	121		
Total or average	8	294	4	325	13	216	11	216	9	188	20	187					8	115	2	105

[a] Based on market classification of Bureau of Agricultural Economics, U. S. Department of Agriculture

EXPERIMENTAL ANIMALS

Arrangements were made to study the chemical composition and cutting yields of hog carcasses selected from miscellaneous experiments conducted during the period 1924–31 by the Department, either independently or in cooperation with the experiment stations of Michigan, Mississippi, North Carolina, and Ohio. Seventy-five of these carcasses were those of hogs used in regular nutrition tests and were analyzed chemically. For 523 additional hogs only the cutting yields were available. The 75 carcasses used in the analysis were from hogs that ranged in weight from 99 to 342 pounds. In this group of hogs, as well as in the entire group of 598 hogs, Poland China, Duroc-Jersey, and Chester White breeds predominated (table 1).

The rations of the 75 hogs contained one or more basal feeds, such as corn, peanuts, hominy, and brewers' rice, and one or more supplements, such as tankage, skim milk, buttermilk, blood meal, alfalfa meal, and minerals. Sixty of these hogs were self-fed or hand-fed on a full-feed basis. The remaining 15 animals were hand-fed at 3 levels of intake, approximately 4, 3, and 2 percent of their live weight to a final weight of about 200 pounds.[5] These 75 hogs were therefore excellent representatives of thrifty hogs handled under varying conditions on different feeds and on different quantities of feed. It was believed that the relationship between their cutting yields and the fat content of the edible portion of the carcass would be representative of that existing among the various kinds of hog carcasses on which this index might be used.

The range in live weight of the 598 hogs used in this study was from 75 to 393 pounds. Distribution among breed and sex is shown in table 1. In the main they were a group of thrifty, well-fed hogs slaughtered at different stages in the fattening process. The number and varied character of these hogs would seem to minimize the effect of individual differences and to make their records a good index of the variation to be expected in cutting yields, among the weights involved.

EXPERIMENTAL PROCEDURE

All the carcasses were handled in the meat laboratory of the United States Animal Husbandry Experiment Farm at Beltsville, Md., according to a routine procedure for dressing, chilling, grading, and separating into cuts. The carcasses were scalded, scraped, split through the center of the backbone, and placed immediately in a cold-storage room, where they were held at a temperature between 34° and 40° F. for approximately 3 days, and then weighed and cut. The cutting method used was selected as the one most likely to produce uniform, comparable fat and lean cuts. As few cuts as possible were made, separations followed anatomical lines when possible, and trimming was standardized and reduced to a minimum.

The method of cutting the carcasses used in these experiments is shown in figure 1. The square-cut head (*e*) (jowls left on) was removed at the occipital joint at right angles to the body. The 3-rib shoulder (*d*) was trimmed just above the knee. The ribs and neck bone were removed, but the plate, or outer layer of fat, was not.

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[5] ELLIS, N.R., and ZELLER, J. H. EFFECT OF QUANTITY AND KINDS OF FEED ON ECONOMY OF GAINS AND BODY COMPOSITION OF HOGS. (Unpublished material.)

About one fourth inch of back fat was left on the loin (*b*). The line separating the loin from the belly was just ventral to the tenderloin muscle (psoas major). The belly (*c*) (spareribs removed) was trimmed through the line of teats, and the brisket, back, and flank end were merely squared. Many of the heavy bellies carried thick fat on brisket and back, but as was the case with the shoulders and plate,

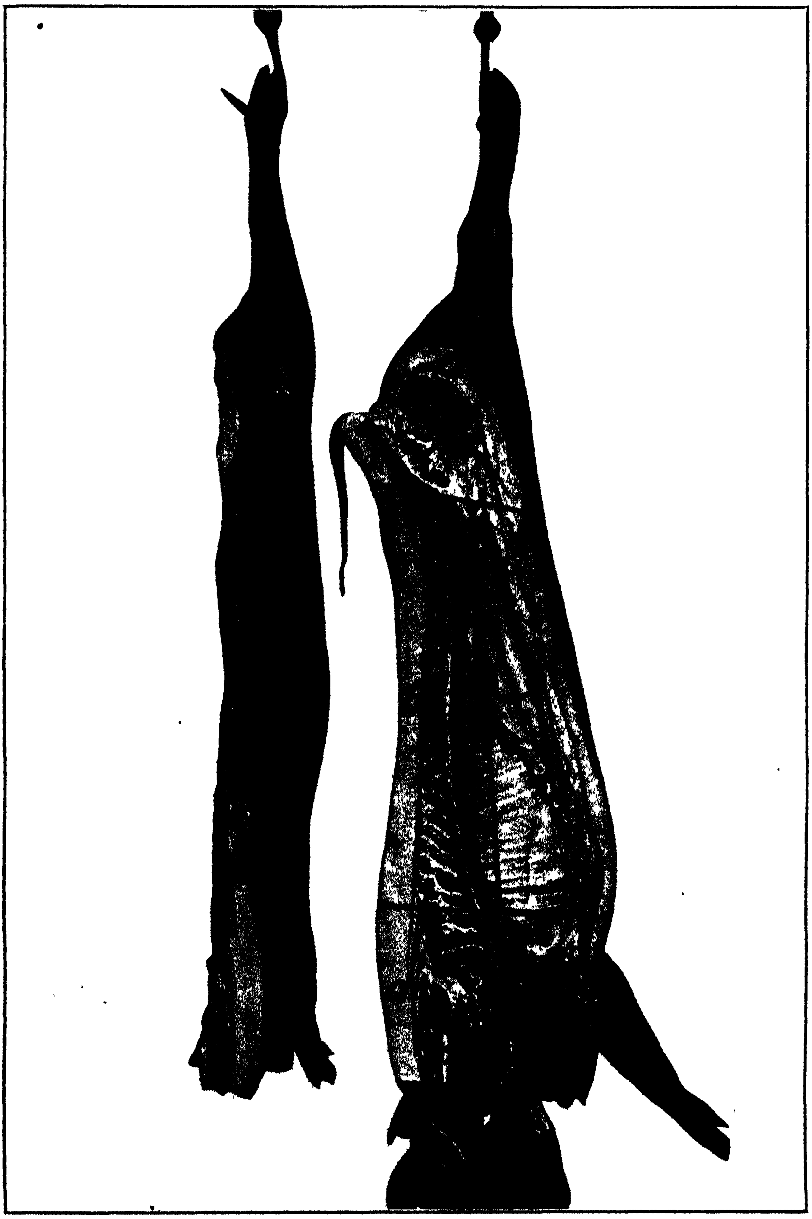

FIGURE 1.—Cuts into which the carcass was divided *a*, Ham, *b*, loin and back fat; *c*, belly; *d*, shoulder; *e*, head, *f*, feet.

it was deemed desirable to give all cuts a comparable trim A short-cut, unskinned ham was removed just behind the second sacral vertebra at right angles to the hind leg. The hind leg was cut off through the hock. All trimmings were combined with the leaf and back fat and were then divided into fat (hereafter called cutting fat), lean trimmings (sausage), and skin. The skin included that obtained from both trimmings and back fat. The method seemed to be the most simple and free from error that could be devised. It was felt that the regular 3-rib unskinned shoulder and square-cut head provided more uniform cuts than the customary separations of the one into picnic, butt, and plate, and the other into face and jowls. Trimming the belly in a uniform manner proved the most difficult task of all. This source of variation was largely removed, however, by combining the weight of the fat trimmings, which included most of the belly trimmings, with the weight of the trimmed belly, in the final calculation for fatness.

After the various parts were separated, trimmed, and weighed, they were boned and skinned. The combined fat and lean parts, constituting the total edible portion, were weighed, ground, mixed, and sampled. The samples were analyzed for fat content by extraction with ether. The fat obtained in this manner was calculated as the percentage of total edible meat.

The percentage yield of the various cuts was determined by dividing the weight of each cut by the weight of the entire chilled carcass. The proportion of chemically analyzed fat in the carcass was determined by using the weight of the cold edible portion as the base.

In selecting the cuts to be used, it was necessary to take those the percentages of which seemed to offer the best probability of serving as an index of actual fatness. A study of these percentages showed that there was a consistently greater percentage yield of cutting fat and belly from carcasses containing a higher proportion of fat in the edible portion. The percentage yield of all the other cuts decreased as the fat content increased. This automatically centered the choice of fat cuts on either cutting fat or belly. It was decided that the percentage yields of the two cuts together would be more representative and free from error than either one alone.

The ham and loin were selected as the cuts most representative of the leanness of the animal. The percentage yield of both showed a consistent inverse relation to the fat content. In addition, these two parts, as cut and trimmed in this study, are standard cuts in all parts of the country, more so than the shoulder and head. There is also less opportunity for inaccuracies in cutting off the ham and loin than in the removal and trimming of the two cuts just mentioned.

Correlations between percentage yields of respective parts and the fat content were made by the simple linear method. Both coefficients of correlation and the regression equations were calculated.

RESULTS AND DISCUSSION

Table 2 shows the average percentage of fat in the edible portion of the carcass, the percentage yields of the fat and lean cuts and their ratios in the 75 chemically analyzed hog carcasses grouped according to weights. The fat content normally ranged from 28 to 59 percent; one exceptional carcass contained 67.9 percent. The range in the combined percentage yields of the 2 fat cuts was from 14.6 to 37.1 percent, and of the 2 selected lean cuts from 29.1 to 37.5 percent. These ranges occurred in an apparently average group of hogs varying in live weight from 99 to 342 pounds.

TABLE 2.—*Comparative content of fat in edible portion of carcass, percentage yield of 2 fat and 2 lean cuts, and their ratio in 75 hog carcasses analyzed chemically*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Average weight of cold carcass	Average fat in total edible portion of carcass	Average percentage of cutting fat [b] and belly [c]	Average percentage of ham and loin [c]	Ratio of percentage yield of 2 fat cuts to that of 2 lean cuts
	Number	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	
Heavy (250 pounds or more)	12	304	241.5	57.3	32.9	30.2	1:1.09
Medium (200–249 pounds)	24	216	170.0	49.5	28.5	32.4	1:0.88
Light (160–199 pounds)	29	187	145.5	45.1	26.2	33.4	1:0.78
Pig (less than 130 pounds)	10	113	81.0	36.3	20.0	33.9	1:0.60

[a] Based on market classification of Bureau of Agricultural Economics, U. S. Department of Agriculture.
[b] Skinless back fat and fat trimmings and the leaf fat.
[c] Based on weight of cold carcass.

The heavy hogs possessed the largest percentage of fat, the largest percentage yield of the 2 fat cuts (cutting fat and belly), and the smallest percentage yield of the 2 lean cuts (ham and loin). In contrast, the lightest group of pigs produced the smallest percentage yield of fat and of fat cuts, and the largest percentage yield of the lean cuts. Comparative percentages for the medium weight and light weight groups varied between the extremes for pigs and heavy hogs in accordance with live weights as can be seen in table 2. The individual records of these hogs, ranked in accordance with the fat content in the edible portion, conformed to the trends of the averages. These results correspond to observations made by other investigators and agree with the increase in fatness expected in a normal group of fattening hogs.

The observed relationship between fat content and the percentage yield of the respective parts establishes those percentages as a possible index of fatness. It now remained to determine that relationship mathematically, by a series of correlations, to calculate the accuracy or error that might be expected from the use of selected cutting yields as a measure of the tendency of hogs to fatten.

MATHEMATICAL RELATIONSHIP BETWEEN FAT CONTENT OF EDIBLE PORTION OF CARCASS AND PERCENTAGE YIELD OF CUTS

The first correlation was between the combined weight of the cutting fat (leaf, skinned fat from trimmings, and skinned back fat) and belly, expressed as a percentage of the weight of the entire cold carcass, and the percentage of fat in the edible portion of the 75 carcasses. The coefficient of correlation expressing that relationship was $+0.91 \pm 0.01$.

This correlation is high. Squaring it gives the coefficient of determination, meaning, in this instance, that approximately 83 percent of the variation in the percentage yield of the two fat cuts was associated with corresponding changes in fat content of the carcass. Therefore it is possible to use the percentage yield of the two fat cuts as a measure or index of the fatness of the carcass or as a base for calculating, with reasonable accuracy, the percentage of fat without actually analyzing the carcass.

A second correlation was made to determine the relationship between the fat content and the percentage yield of the trimmed belly combined with that of only the unskinned back fat instead of all the cutting fat. This was done to provide an even more simple index in which only the belly and back fat would have to be cut by the prescribed method. All the other cuts could then be made and trimmed according to the local preference prevailing in the packing houses where the test hogs might be handled. The coefficient expressing the correlation between fat content and the percentage yield of belly and unskinned back fat was +0.84 ± 0.02, with a corresponding coefficient of determination of 71.

The third correlation was between the combined weight of the two selected lean cuts, trimmed ham and loin, expressed as a percentage of the entire cold carcass, and the fat content of the edible portion of the carcass. The coefficient expressing that relationship was −0.77 ± 0.03, with a coefficient of determination of 59.

The fourth and final correlation was between the fat content and the ratio between the percentage yield of the 2 fat and the 2 lean cuts. The ratio between the percentage yield of the fat and lean cuts of the 75 individual hogs was derived by dividing the percentage yield of the 2 fat cuts by that of the 2 lean cuts. The same result could have been obtained by dividing the actual weights of the selected parts. This ratio, shown in table 2, varied from an average of 1.09 for the heavy hogs to 0.60 for the pigs and expressed the same relationship noted by the German investigators (*6*) who used a slightly different set of cuts. The coefficients of correlation between the ratio and the fat content was +0.92 ± 0.01 with a coefficient of determination of 85.

The results obtained from all these correlations indicate a high relationship between changes in the actual fatness of a hog and changes in the percentage of its various parts or cuts. The ratio between the percentage of the 2 fat and the 2 lean cuts appears as a slightly more accurate index of fatness than does the percentage of cutting fat and belly. The coefficient of correlation for the ratio of fat to lean cuts was slightly higher, +0.92 ± 0.01 as compared with +0.91 ± 0.01, but this difference is negligible. Both ratios are considerably more reliable as indexes of fatness than the percentage yield of the belly and back fat alone (coefficient of correlation +0.84 ± 0.02) or the percentage yield of trimmed ham and loin (coefficient of correlation −0.77 ± 0.03).

The combined percentage yield of the cutting fat and belly was selected as a more practical and easily obtained index than the ratio.

The use of the percentage yield of the two fat cuts as an index of fatness seems to apply with equal accuracy to hogs of various weight (fig. 2). About two thirds of each of the weight groups plotted on that scatter graph fall within the standard error of estimate.

The proposed index also applies with almost equal accuracy to both barrows and gilts. A correlation between the index and the fat content in the edible portion of the 38 barrow carcasses developed a coefficient of +0.92 ± 0.02. A similar coefficient for the 37 gilts was +0.90 ± 0.02.

In view of the consistent relation between the proposed index and the fatness of the carcasses, a regression equation was developed for calculating the fat content of the edible portion of the carcass from the percentage yield of the cutting fat and belly. Thus:

$$Y = 5.57 + 1.54X$$

in which Y is the percentage of fat in the edible portion, and X is the fat index or percentage yield of cutting fat and belly. The regression line of this equation indicates that for each increase of 1 in the percentage yield of the two fat cuts there is a corresponding increase of 1.54 percent of fat in the edible portion of the carcass (fig. 2).

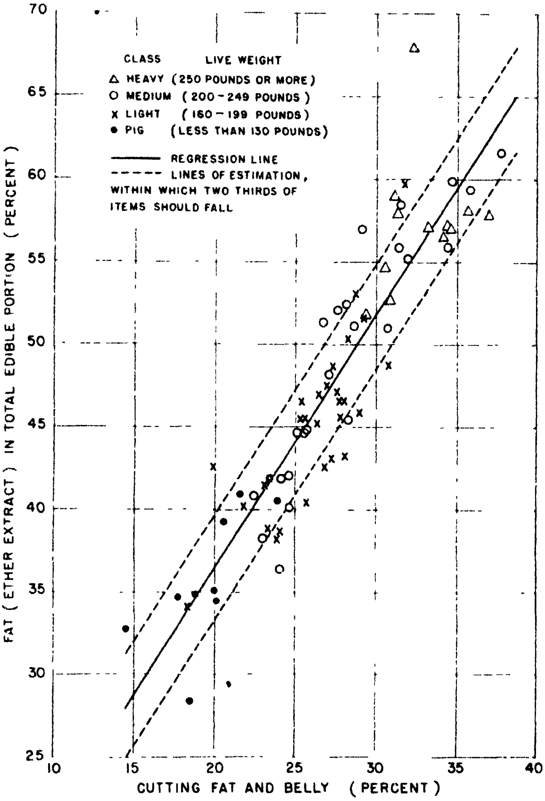

FIGURE 2.—Variation in percentage of fat (ether extract) in the total edible portion associated with variation in yield of cutting fat (fat from skinned back fat, skinned trimmings, and leaf) and belly in carcasses of 75 hogs weighing from 99 to 342 pounds alive.

A second regression equation is included, using as the index the percentage yield of the belly combined with that of only the unskinned back fat. This provides a means of calculating fatness at those times or places where commercial methods make it impossible to cut the entire carcass. It should be remembered that the coefficient of correlation between fat content and the percentage yield of belly and back fat was only +0.84 ± 0.02. This provides a fair estimate of fatness but is less accurate than the equation based on the correlation between fat content and the percentage yield of belly and cutting fat.

The regression equation for calculating the fat content of the edible portion of the carcass from the percentage yield of belly and unskinned back fat is

$$Y = 7.11 + 2X$$

in which Y is the percentage of chemically determined fat in the edible portion and X is the percentage yield of belly and unskinned fat. The regression line for this equation indicates that for each increase of 1 in the percentage yield of belly and back fat there is an increase of 2 in the percentage of fat.

The fat content of the carcass, as calculated by the use of these two equations from the percentage yield of the fat cuts, is given in table 3. This table shows that for each 5-percent increase in the actual percentage yield of fat cuts there is a greater increase in the percentage of fat in the carcass when calculated from back fat and belly than when calculated from cutting fat and belly.

TABLE 3.—*Percentage of fat in carcass calculated from actual percentage yield of cutting fat and belly and of back fat and belly*

Actual yield of cutting fat and belly	Fat[a] in carcass calculated from percentage of cutting fat and belly	Actual yield of back fat and belly	Fat[b] in carcass calculated from percentage of back fat and belly
Percent	*Percent*	*Percent*	*Percent*
15	28.6	10	27.1
20	36.3	15	37.1
25	44.0	20	47.1
30	51.7	25	57.1
35	59.3	30	67.1

[a] Standard error of estimate is ±3.3

[b] Standard error of estimate is ±4.1

USE OF THE INDEX IN COMPARING FATNESS IN HOGS

The ranges in the index were then used to classify the live-weight groupings of all the 598 hogs considered in this study, as given in table 4. These hogs included the 75 that were analyzed chemically. The representative character of these hogs (table 1) and the comparative percentage yields of their fat, lean, and other cuts (table 4) would indicate that they were normal fattening hogs.

TABLE 4.—*Average percentage yields of various parts of 598 hog carcasses classified according to live weight*

Classification of hogs[a]	Hogs, analyzed	Average live weight at slaughter	Average weight of cold carcass	Shrink in chilling[b]	Selected fat cuts[c]			Selected lean cuts[c]		
					Cutting fat[d]	Belly	Total (fat index)	Ham	Loin	Total
	Number	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy (250 pounds or more)	92	289	238.5	2.42	21.5	11.9	33.4	17.0	11.0	28.0
Medium (200–249 pounds)	240	218	177.5	2.81	19.5	11.4	30.9	17.5	11.6	29.1
Light (160–199 pounds)	199	183	146.0	2.90	16.8	10.8	27.6	18.4	12.1	30.5
Light-light (130–159 pounds)	41	146	116.0	2.89	15.0	10.1	25.1	18.4	12.2	30.6
Pig (less than 130 pounds)	26	106	78.0	3.04	10.9	9.4	20.3	19.9	13.2	33.1

Classification of hogs[a]	Other cuts or parts								Shrink in cutting
	Shoulder	Head	Spare ribs	Shoulder ribs	Sausage	Feet	Skin	Kidney and tail	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy (250 pounds or more)	16.7	8.5	1.9	1.8	4.3	2.4	2.3	0.4	0.3
Medium (200-249 pounds)	17.0	8.9	2.0	2.0	4.3	2.7	2.4	.5	.2
Light (160–199 pounds)	17.4	9.4	2.1	2.2	4.6	3.0	2.4	.5	.3
Light-light (130–159 pounds)	17.7	9.8	2.3	2.5	5.3	3.3	2.5	.6	.3
Pig (less than 130 pounds)	18.5	10.7	2.6	2.7	5.1	3.9	2.5	.5	.1

[a] Based on market classification of Bureau of Agricultural Economics, U.S. Department of Agriculture.

[b] Percentage based on weight of hot carcass immediately after slaughter.

[c] Percentage based on weight of cold carcass.

[d] Skinless back fat and fat trimmings and the leaf fat.

In order to show the relationships between the proposed index, or percentage yield of cutting fat and belly, and the market grades and relative fatness of hogs associated with such grades, the following classification based on percentage yield of cutting fat and belly is suggested:

Very lean, 22 percent or less.
Lean, 22.01 to 26 percent.
Moderately fat, 26.01 to 30 percent.
Fat, 30.01 to 34 percent.
Very fat, 34.01 percent or more.

The suggested fat classification applied to these 598 hogs places the 92 heavyweight hogs near the top and the 240 mediumweight hogs near the bottom of the fat class with fat indexes of 33.4 and 30.9, respectively (table 4). The 199 lightweight hogs are classified as moderately fat, the 41 light-light hogs in the top of lean, and the 26 pigs as very lean. This classification seems to be a fair statement of the degree of fatness of the hogs. It is therefore offered as a starting point in the effort to compare accurately the tendency of hogs to form fat and lean.

The effect of sex on degree of finish in hogs offers an excellent example of the way the proposed fat index may be used. The records of 523 hogs (the ones not included in the correlations) have been classified by live weight and sex in table 5. Although the average live weight for both barrows and gilts is similar, the barrows show a consistently greater percentage yield of the two fat cuts. Differences in the heavyweight group are less than in the others.

TABLE 5.—*Comparative fat index and percentage yield of ham and loin from car-*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Total percentage yield of cutting fat [b] and belly (fat index) [c]	Total percentage yield of ham and loin [c]	Ratio of percentage yield of 2 fat cuts to that of 2 lean cuts
Heavy (250 pounds or more).	*Number*	*Pounds*	*Percent*	*Percent*	
Barrow	48	255	33.6	27.5	1:1.22
Gilt	32	290	33.3	28.0	1:1.19
Medium (200–249 pounds).					
Barrow	126	218	31.7	28.2	1:1.12
Gilt	90	219	30.2	29.5	1:1.02
Light (160–199 pounds):					
Barrow	79	182	29.0	29.3	1:0.99
Gilt	91	183	26.8	30.6	1:0.88
Light-light (130–159 pounds).					
Barrow	19	146	26.0	30.0	1:0.87
Gilt	22	146	24.2	31.2	1:0.78
Pig (less than 130 pounds):					
Barrow	9	104	21.2	31.8	1:0.67
Gilt	7	100	19.5	32.9	1:0.59

[a] Based on market classifications of Bureau of Agricultural Economics, U. S. Department of Agriculture.
[b] Skinless back fat and fat trimmings and the leaf fat.
[c] Based on weight of cold carcass.

The percentage yield of the lean cuts and of the ratio between percentage yield of fat and lean cuts are included to show that they, as well as the selected index, indicate that barrows fatten more rapidly than gilts.

The indicated greater fatness of the barrows reverses the general trend of sex in cattle, in which the heifers usually fatten more quickly than the steers.

In view of the greater fatness of the barrows, as shown by this index, it is of interest to recall Russell's findings *(5)* with respect to gain. His records cover a period of 8 years and include the gains of 5,653 hogs, of which 3,018 were barrows and 2,635 gilts. The gains in weight of the barrows, Russell found, were 5.43 percent greater than those of the gilts.

The fat index offers a means of recording the fatness of litters produced and tested in breeding or record-of-performance trials. Though more subject to variation when applied to small groups of hogs, it provides a method for recording the differences between litters and the progress or change resulting from planned matings.

The fat index can be used also in classifying the fatness of individual hogs, as illustrated in a special test made to determine the dietetic value of the cuts from a very thin hog of so-called "market live weight." The carcass selected and cut was from a Chester White gilt (no. 31.4) weighing 167 pounds alive. Her fat index (percentage yield of cutting fat and belly) was only 21.5. According to the arbitrary classification suggested in table 4 this would place her among the very thin hogs. This index is compared in table 6 with those of hogs of greater and of less live weight. Although this hog weighed 167 pounds alive, her fat index classifies her as having a finish comparable to that of the group averaging less than 130 pounds live weight.

Table 6.—*Fat index of hog no. 31.4 compared with index of light, light-light, and pig weight groups*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Average weight of cold carcass	Fat index [b]
	Number	*Pounds*	*Pounds*	
Light (160-199 pounds live weight)	199	183	146	27.6
Light-light (130-159 pounds live weight)	41	146	116	25.1
Hog no. 31.4	1	167	132.5	21.5
Pig (less than 130 pounds live weight)	26	106	78	20.3

[a] Based on market classifications of Bureau of Agricultural Economics, U. S. Department of Agriculture

[b] Percentage yield of fat from skinned trimmings and back fat, leaf, and belly, based on weight of cold carcass

The fat content of the total edible portion of this hog carcass, as estimated from her fat index by the formula used for making the estimations of fat contents in table 3, was 38.68 percent ± 3.3, also within the fatness range of the pig group (less than 130 pounds live weight). The actual fat content, as later determined by chemical analysis (table 8) was 37.29 percent, which was well within the standard error of estimate.

In contrast with 37.29 percent of fat in the total edible portion of this lean pork carcass is the fat content of beef sides reported by Chatfield *(2)*. She classifies sides containing from 25 to 35 percent of fat in the total edible portion as fat, and those containing 35 percent or more as very fat.

Both physical and chemical analyses of the cuts from this one hog were made. The percentage yields of total edible and inedible meat, lean, fat, bone, and skin are shown in table 7. The proportion of total edible meat in the major cuts from this hog ranged from 76.38

percent in the loin to 92.10 percent in the belly. The proportion of physically separable fat from the major cuts ranged from 10.63 percent in the loin to 36.81 percent in the belly. The yield of lean was approximately 1.9 times the yield of fat in the total carcass. This is comparable to a value of 1.7 for the lean to fat relationship in 5 hogs fed only a 2-percent grain ration until they reached a weight of 200 pounds. These 5 hogs are included in the 75 hogs analyzed chemically. Another group of 5 hogs fed a full ration (4 percent of live weight) had approximately 1.1 times as much lean as fat in the carcasses.

TABLE 7.—*Physical composition of carcass and cuts from hog no. 31.4 weighing 167 pounds and with a low fat index of 21.5*

Cut	Weight of cut	Edible and inedible content		Physical composition			
		Total edible	Total unedible	Lean	Fat	Bone	Skin
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Butt [a] (top third of shoulder)	8.54	100.00		88.17	11.83		
Leaf fat	2.58	100.00			100.00		
Belly	12.66	92.10	7.90	55.29	36.81		7.90
Trimmings [b] (excluding back fat, plate, and leaf)	14.11	88.45	11.55	49.47	38.98		11.55
Clear plate [a] (outside fat from butt)	3.50	87.42	12.57		87.42		12.57
Shoulder (regular 3-rib)	25.86	85.50	14.50	62.61	22.89	10.63	3.87
Back fat	9.31	83.24	61.76		83.24		16.76
Ham	24.94	82.72	17.28	66.68	16.04	11.79	5.49
Picnic [a] (shank end of shoulder)	12.63	79.26	20.74	64.61	14.65	16.31	4.43
Loin	18.25	76.38	23.62	65.75	10.63	23.62	
Spareribs	3.07	53.09	46.91	53.09		46.91	
Head, including tongue	13.05	49.27	50.73	32.57	16.70	34.48	16.25
Shoulder ribs	2.90	31.03	68.97	31.03		68.97	
Fore feet	2.37		100.00			52.74	47.25
Hind feet	2.31		100.00			59.31	40.69
Kidney and tail	.88	40.91	59.09	40.91		34.09	25.00
Total carcass [c]	132.29	75.67	24.33	49.57	26.10	15.94	8.39

[a] Made from 3-rib shoulder

[b] Does not include trimmings from butt and picnic

[c] Exclusive of butt, clear plate, and picnic, which were cut from the shoulder.

Table 8 shows the chemical composition of the various cuts and of the total edible meat in the carcass of hog no. 31.4. The arrangement of the cuts according to increasing fat content of the edible portion shows the group comprising the lower half to include the loin, butt, ham, picnic, and the 2-rib cuts. The separable lean portion of the ham contained 6.83 percent and the lean of the loin only 9.45 percent of fat. Although the fat content of the total edible meat in the carcass of this hog was low in comparison with the usual values on fat hogs, the fat content of the separable lean portions was not reduced proportionately. This is shown by comparison with analyses on the meat from the hogs on the 2- and 4-percent feeding levels[6] already mentioned. These results showed a comparatively small decrease in fat content, with decrease in feed level, in the lean of the ham and the total edible portion of the trimmed loin as compared with the marked changes in the fat content of the total edible portion of the ham and the entire carcass. The fat content of each of the last two items for the lot on the low-feeding level (2 percent of live weight) was within 1 percent of the corresponding values on hog 31.4, whereas the lean of the hams from the full-fed (4 percent) lot had a fat content of 6.22 percent, or less than that given in table 8.

ELLIS, N. R., and ZELLER, J. H. See footnote 5.

TABLE 8.—*Chemical composition of carcass and cuts from hog no. 31.4*

Cut	Weight of total edible portion	Chemical composition of edible portion				Weight of separable lean	Chemical composition of separable lean			
		Protein [a]	Fat [a] (ether extract)	Ash [a]	Water [a]		Protein [b]	Fat [b] (ether extract)	Ash [b]	Water [b]
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*
Loin	13.94	17.68	17.25	0.93	64.53	12.00	19.20	9.45	1.01	70.69
Butt [c] (top third of shoulder)	8.54	16.24	20.10	.87	63.34	7.53	([d])	----	----	----
Ham	20.63	16.58	20.99	.88	61.91	16.63	19.48	6.83	1.05	72.58
Picnic [c] (shank end of shoulder)	10.01	16.27	22.14	.83	61.50	8.16	18.27	11.20	.95	69.36
Spareribs	1.63	16.59	22.70	.91	59.87	1.63	([d])	----	----	----
Shoulder rib	.90	15.92	25.57	.45	57.86	.90	([d])	----	----	----
Shoulder (regular 3-rib)	22.11	14.46	30.14	.76	55.40	16.19	17.94	12.20	.95	70.45
Head, including tongue	6.43	11.50	39.70	.64	48.14	4.25	([d])	----	----	----
Belly	11.66	11.69	43.95	.62	44.73	7.00	16.48	19.71	.86	63.59
Trimmings [e] (excluding back fat, plate, and leaf)	12.48	9.66	54.15	.44	36.52	6.98	14.44	30.35	.62	54.73
Back fat and clear plate [c] (outside fat of butt)	10.81	3.60	84.34	.21	13.41	----	----	----	----	----
Leaf fat	2.58	2.50	91.58	.11	7.72	----	----	----	----	----
Total edible in carcass	100.11	13.13	37.29	.68	49.57	65.58	18.00	13.45	.93	68.17

[a] Based on total edible in each cut.
[b] Based on separable lean in each cut.
[c] Made from 3-rib shoulder.
[d] Composite sample of lean and fat.
[e] Does not include trimmings from butt and picnic.

These data suggest that the lean meat of hog 31.4 was similar in composition to that of hogs of moderate fatness.

SUMMARY

Experiments with a large number of hogs of several breeds showed that there was a consistent relationship between the content of fat in the edible portion of the hog carcass and the percentages that the weights of certain cuts bear to the carcass weight. The percentage yield of the fat cuts (belly, leaf fat, and skinned back fat and trimmings) increased with an increase in fat content. The coefficient of correlation expressing that relationship was +0.91 ± 0.01. When the fat cuts consisted of only the belly and the unskinned back fat, the correlation between that percentage and the content of ether extract was + 0.84 ± 0.02.

The combined weight of the belly, leaf, and skinned back fat and trimmings, expressed as a percentage of the cold-carcass weight, is offered as a simple and practical index of the fatness of hog carcasses. This index ranges from 15 to 35 percent and includes carcasses that can be classed as very lean and very fat.

The percentage yield of belly and unskinned back fat is also offered as a practical but less reliable index of fatness for use when the weight of the other fat cuts cannot be obtained.

These indices are proposed for use by hog breeders in determining the tendency of specific types, strains, or selected progeny to form fat or lean.

This fat index, applied to data on 281 barrows and 242 gilts, showed the former to be somewhat fatter. It was also used in a detailed study of a relatively lean hog carcass, the various retail cuts of which had been separated into fat and lean and analyzed physically and chemically.

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PHYSICAL CHARACTERISTICS OF HOG CARCASSES AS MEASURES OF FATNESS[1]

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INTRODUCTION

The fatness of meat animals and carcasses, known also as "finish", is a characteristic of great practical importance. In animals intended for slaughter, fatness merits consideration with respect to both the growing of meat animals and the requirements of consumers. The fattening of an animal is relatively expensive in contrast with producing earlier growth. Normally the consumption of feed per unit of gain during fattening is greater than during the growth period, when the increase in weight consists largely of bone and muscle tissue. The longer the fattening is continued the more costly the gains become. Within a reasonable limit, increased finish is believed by many to be associated with increased desirability of meat from the consumer's standpoint. However, the exact degree of finish associated with the most desirable quality of the different classes of meat and the specific manner in which the fat influences palatability are yet to be determined. From these considerations it is clear that a simple means of measuring fatness, besides being of practical importance, should facilitate technical studies relating to livestock production and the characteristics of meat and meat food products.

In view of the foregoing it is apparent that an essential phase of studies dealing with the quality of meat is the determination, or at least a reliable estimation, of the proportion of fat in the individual carcass Accurate interpretation of results is difficult, if not impossible, without information of this nature. Existing methods for measuring the fatness of hog carcasses may be criticized either as lacking in exactness or as being relatively expensive in time or material.

Any advance in methods for making estimates of fatness is of interest and value to research workers. If the improved method is one which can be applied quickly with little expense, it may have direct value from certain practical points of view.

Experiments conducted as a part of the national project, cooperative meat investigations, have taken carcass composition extensively into account. This has been true particularly in the beef and pork studies. In connection with this work many physical and chemical analyses of beef and pork carcasses and their respective cuts have been made by the Animal Husbandry Division of the United States Department of Agriculture. The ninth–tenth–eleventh rib cut from

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the right side was adopted as the standard beef-carcass sample by the cooperators in the national project, and it has been used to indicate the composition of the dressed carcass as a whole. Through the analyses of a large number of pork carcasses and their respective cuts the Division established the fact[3] that the trimmed ham is a satisfactory sample from which the fatness of the carcass may be estimated. This greatly simplifies the work of physical and chemical analyses of the fat content of hog carcasses.

Cutting yields have been found by Warner, Ellis, and Howe[4] to be a valuable method for estimating fatness. The correlation between the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimmings, and the percentage of ether extract in the total edible portion of the carcass was +0.91 ± 0.02 for a total of 75 hogs. In employing this method of estimating fatness, the carcass, of course, must be cut by a certain standard method and the various cuts weighed. The present paper gives a method which is applicable to the uncut carcass and thus has a somewhat different field of usefulness.

METHODS AND MATERIALS

For several years the Animal Husbandry Division has been interested in the possibilities offered by carcass measurements in connection with meat studies. These included, among other considerations, their possible usefulness in estimating fatness.

In 1929 a set of 15 measurements was developed for use on hog carcasses.[5] This method is applicable to center-split carcasses. A steel tape is used for taking all measurements except nos. 1, 13, and 14. All measurements are taken in millimeters, with the carcass hanging from a hook in the normal position, head down.

METHOD OF MEASURING CARCASSES

LENGTH

(1) Head: From the snout, between nostrils, to the tip of the atlas joint (occipito-atloid articulation). (Calipers.)

(2) Neck: From the base of the atlas joint to the anterior aspect of the first dorsal vertebra.

(3) Body: From the anterior edge of the first rib to the lowest point (as the carcass hangs on the hook) of the aitch bone.

(4) Hind leg: From the lowest point of aitch bone to the coronary band of the foot.

DEPTH AT SEVENTH DORSAL VERTEBRA

(5) Thickness of back fat, exclusive of skin.[6]

(6) Distance from the lower margin of the back fat to the upper edge of the spinal canal.

(7) Distance from the upper edge of the spinal canal to the lower edge of the split breastbone.

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[3] Unpublished data.

[4] WARNER, K. F., ELLIS, N. R., and HOWE, P. E. CUTTING YIELDS [illegible] HOGS AN INDEX OF FATNESS. Jour. Agr. Research 48: 241-255, illus.

[5] This method of measuring hog carcasses, except the steps relating to thickness of back fat, was developed by a committee consisting of O. G. Hankins, H. C. McPhee, and K. F. Warner, of the Animal Husbandry Division. The back-fat measurements had been employed since 1927 by N. R. Ellis, W. O. Pool (resigned), and R. M. Riemenschneider in studies on the relationships between certain production factors and thickness of back fat, and between the latter and firmness as determined by laboratory tests and committee judgment.

[6] Also included with (8), (9), (10), and (11) as one of the 5 measurements of thickness of back fat.

THICKNESS OF BACK FAT

(8) At the first dorsal vertebra.

(9) At point 7 vertebrae below last lumbar. (Include last lumbar vertebra in count.)

(10) At point 3½ vertebrae below last lumbar. (Include last lumbar vertebra in count.)

(11) At last lumbar vertebra.

CIRCUMFERENCE OF FRONT LEG

(12) Right front leg: At point of least circumference between knee and pastern joints.

WIDTH AND PLUMPNESS

(13) Shoulder: Width from inside of carcass at first dorsal vertebra to outside of shoulder on a line parallel to the floor. Sum of measurements of both sides of carcass is recorded. (Calipers.)

(14) Ham: Width from top point of the aitch bone to the outside of the ham on a line parallel to the floor. Sum of measurements of both sides of carcass is recorded. (Calipers.)

(15) Ham plumpness: (*a*) Length from lowest point of aitch bone to center of inside of hock joint located at bony projection which may be felt under the skin; (*b*) circumference at mid point of measurement (*a*), obtained by locating 3 or 4 points on ham equidistant from plane through center of hock joint, such points marked with sharp metal skewers and ham encircled with steel tape immediately below skewers for measurement; (*c*) measurement (*b*) multiplied by 100 and divided by measurement (*a*) gives index of plumpness.

Measurements 5, 8, 9, 10, and 11 are averaged to give the "average thickness of back fat." Detailed consideration is given later to this carcass characteristic.

Since 1929 the foregoing method has been applied to a large number of individual hogs. The carcasses measured were those of animals used in a number of cooperative and independent experiments of varied nature and objectives. Some of the experiments involved chemical analyses of entire carcasses exclusive of bone and skin. Thus data were obtained from which it was possible to determine the relationships between certain selected measurements and the fatness of the edible portion of the carcasses. The purpose of this paper is to report on the relationships found.

Sixty hogs were used in five experiments. Two of the experiments were conducted cooperatively by the North Carolina Agricultural Experiment Station and the United States Department of Agriculture. The others were conducted independently by the Department. The hogs in these experiments varied with respect to age, breed, sex, type, and initial weight, kind and quantity of feed, rate of gain, total gain, final weight, and market grade. The range of final feed-lot weights, for example, was from 93 to 250 pounds. Some of the hogs were fed such a limited ration during part of the experiment as to result in a material loss in weight after they had reached a weight of approximately 225 pounds. The large number of these variables and the extent of the variability in certain instances are believed to add significance to the results of the special study on fatness.

The thickness of back fat is usually observed in forming an opinion on the fatness of a hog carcass and is generally regarded as a dependable, practical guide. As previously explained, the thickness of back fat was measured at five different points, in the work reported herein, and these five measurements on each carcass were averaged, the figure so obtained being designated the "average thickness of back fat." These average values for the 60 hogs were correlated with the percentages of fat (ether extract) in the total edible portion of the carcasses.

RELATION BETWEEN THICKNESS OF BACK FAT AND PERCENTAGE OF FAT IN EDIBLE PORTION OF CARCASS

Figure 1 is a scatter diagram showing the relationship between the average thickness of back fat in millimeters and the percentage of fat in the total edible portion of the carcass, for the 60 hogs analyzed. The regression line and its standard error are also shown.

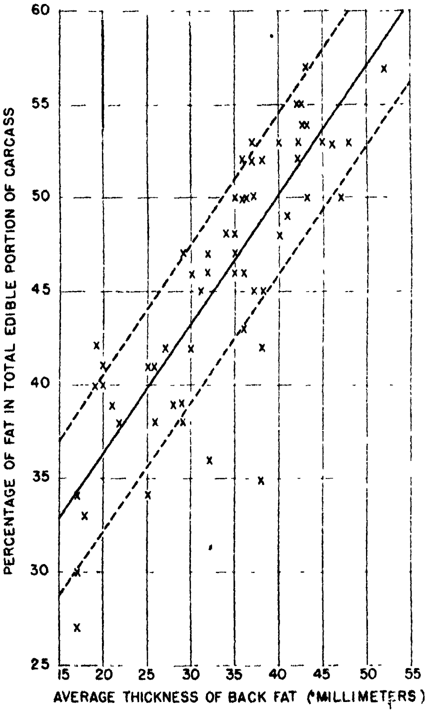

FIGURE 1.—Relation between average thickness of back fat and percentage of fat in edible portion of hog carcass. Each symbol (X) represents 1 of 60 hogs varying in type.

The equation for the regression line, of the form $y = a + bx$, is as follows:

Percentage of fat in total edible portion of carcass = 22.45 + 0.691 × average thickness of back fat.

It appears from figure 1 that the relationship is linear or that the straight line fits the points on the diagram in a satisfactory manner. It does not necessarily hold, however, that this relationship would apply to hogs having back fat less than about 15 mm in thickness, in which case the trend would obviously be downward. The standard error of the regression line or of the percentage of fat in the edible portion of the carcass is 4.2.

The correlation coefficient representing the relationship between the two characteristics under consideration was found to be + 0.84 with a standard error of ± 0.04. The corresponding coefficient of determination is 0.71. The relationship, represented by the coefficient + 0.84, was the closest found between fatness and any of the other physical characteristics of the carcasses included in the study of the 60 hogs as one group.

It is of interest in this connection that the correlation between the chemically determined fat content of the edible portion of the carcasses and of the trimmed, right hams, the latter being the standard carcass sample for analysis, was + 0.93 ± 0.02. A comparison of the square (0.86) of this coefficient with 0.71, mentioned above, indicates that slightly greater accuracy is gained by analyzing the ham, instead of measuring the thickness of back fat to obtain an estimate of the fatness of the carcass. Conversely, the comparison indicates the accuracy sacrificed by employing the more rapid, less expensive method which may be applied as the split carcasses hang on the rail.

In the study by Warner, Ellis, and Howe[7] the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimming, and the percentage of fat in the total edible portion of the hog carcass showed a relationship represented by the correlation coefficient +0.91 ± 0.02. The standard error of the regression line or in the percentage of carcass fat as estimated by this cutting-yield method was ± 3.3. It is apparent from these results that the average thickness of back fat is a hog-carcass characteristic of very definite value for estimating the fatness of the edible portion of the carcass as a whole.

TYPE OF HOG IN RELATION TO VALUE OF THE METHOD

With a few exceptions the type as shown by the individual hogs just prior to slaughter was judged and recorded by a committee of three members of the Department staff.[8] Three major types were recognized, viz, large, intermediate, and small. Thirty-four of the hogs were classed as intermediate in type. This relatively large number of one type afforded an opportunity to throw light on the question whether average thickness of back fat is a more useful index of fatness when type is uniform than when it varies.

Figure 2 shows the relationship between the two factors among the 34 intermediate-type hogs.

The equation for the regression line representing the 34 hogs is as follows:

Percentage of fat in total edible portion of carcass = 25.16 + 0.621 × average thickness of back fat.

The standard error of the regression line is ± 3.5. This may be compared with ± 4.2 when all 60 hogs are considered. By assuming an infinite number of hogs in both cases and adjusting the standard errors of the regression lines accordingly, the difference between the standard errors was found to remain practically the same. The smaller standard error of the regression line for the 34 hogs suggests that uniformity in type was a factor resulting in slightly greater accuracy in the estimation of percentage of fat in the edible portion of the carcass from the average thickness of back fat. However,

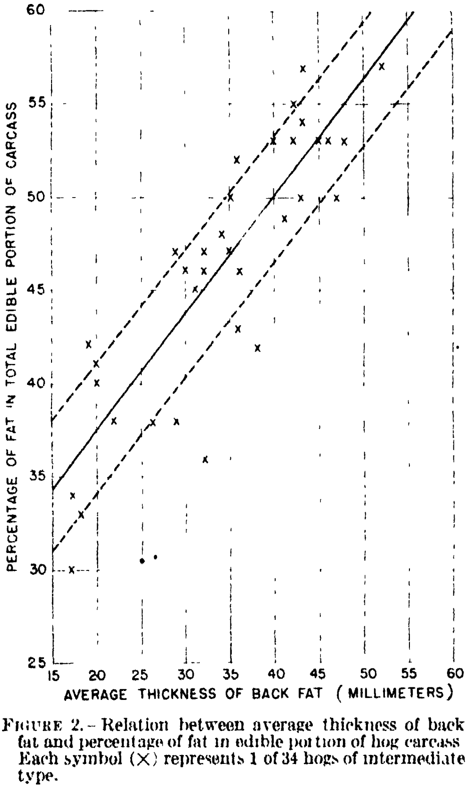

FIGURE 2.—Relation between average thickness of back fat and percentage of fat in edible portion of hog carcass Each symbol (X) represents 1 of 34 hogs of intermediate type.

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[7] WARNER, K. F., ELLIS, N. R. and HOWE, P. E. See footnote 4.

[8] Two members of this committee were representatives of the Bureau of Animal Industry, and one of the Bureau of Agricultural Economics.

the corresponding correlation coefficients were +0.87±0.04 and +0.84±0.04, and the difference between the two is not statistically significant.

Table shows the average calculated percentages of fat in the edible portion of the carcass which correspond to different thicknesses of back fat. It was prepared by using the regression equation given above.

TABLE 1.—*Average calculated percentages of fat in edible portion of hog carcasses as related to different thicknesses of back fat*

[Average of 5 measurements in each case]

Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—		Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—		Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—	
	Hogs varying in type	Hogs intermediate in type		Hogs varying in type	Hogs intermediate in type		Hogs varying in type	Hogs intermediate in type
	Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
0	29.1	31.4	25	39.7	40.7	40	50.1	50.0
5	32.8	31.5	30	43.2	43.8	45	53.5	53.1
0	36.3	37.6	35	46.6	46.9	50	57.0	56.2

Table 1 shows that at a back-fat thickness of 40 mm the difference in fatness between the two groups of hogs was least.

OTHER FACTORS CONSIDERED IN RELATION TO FATNESS

A number of other measurements, in addition to average thickness of back fat, were studied to determine their values as indices of fatness or of the percentage of fat in the total edible portion of the carcass. Certain ratios, final feed-lot weight, and chilled-carcass weight were also considered. The relationships found are shown below in decreasing order of magnitude, expressed as coefficients of correlation. The standard error of the coefficient is also shown in each instance.

(1)	Thickness of back fat at seventh dorsal vertebra (measurement 5)	+0.77±0.05
(2)	Width through shoulders (measurement 13)	+ .74± .06
(3)	Weight of chilled carcass divided by total length of body and leg (sum of measurements 3 and 4)	+ .73± .06
(4)	Thickness of back fat at seventh dorsal vertebra (measurement 5) divided by depth of carcass exclusive of back fat (sum of measurements 6 and 7)	+ .72± .06
(5)	Weight, final feed-lot	+ .67± .07
(6)	Weight of chilled carcass	+ .67± .07
(7)	Depth of carcass (sum of measurements 5, 6, and 7) divided by length of leg (measurement 4)	+ .65± .07
(8)	Width through hams (measurement 14)	+ .63± .08
(9)	Plumpness of ham (measurement 15)	+ .59± .08
(10)	Length of body (measurement 3) divided by mean of widths through shoulders and hams (measurements 13 and 14)	− .58± .09
(11)	Depth of carcass (sum of measurements 5, 6, and 7)	+ .56± .09
(12)	Length of body (measurement 3) divided by depth of carcass (sum of measurements 5, 6, and 7)	− .54± .09
(13)	Length of body (measurement 3)	+ .40± .11
(14)	Depth of loin (measurement 6)	+ .39± .11
(15)	Entire length (sum of measurements 1, 2, 3, and 4)	+ .35± .11
(16)	Length of ham (measurement 15)	+ .33± .12

Consideration of these 16 correlation coefficients is simplified by classifying them into four groups. Group 1 includes those which involve direct measurement of the layer of external fat or coefficients

(1, 2, and 8). There is little difference between the thickness of back fat at the seventh dorsal vertebra and the width of carcass through the shoulders as indices of percentage of fat in the total edible portion of the carcass. However, width through the hams is represented by a considerably lower value.

Group 2 includes all coefficients which involve a ratio as one of the correlated factors. These are the relationships designated above as 3, 4, 7, 9, 10, and 12. The first-mentioned and highest, in which weight per unit of length is considered in relation to fatness, is a moderately high value (+0.73±0.06). The second is practically identical with it. Relationship 9 indicates that increasing plumpness of ham is not necessarily closely accompanied by increasing fatness of carcass. This suggests, in turn, that a high degree of plumpness may be due largely, in some cases, to unusual muscular development without a thick covering of fat. Relationships 10 and 12, the two lowest in this group, are interesting on account of the small difference between them. It is obvious that it made little difference whether the average of the widths of carcass through the shoulders and hams or the depth of carcass was involved in the ratio.

Group 3 consists of the relationships which involve weight as one of the correlated factors. The two such relationships included are those involving (1) final feed-lot weight (5), and (2) chilled-carcass weight (6). The correlation coefficients and standard errors in the two cases are identical (+0.67±0.07). The corresponding coefficient of determination, 0.45, indicates that slightly less than 50 percent of the variation in fat content of the edible portion of the carcass is associated with weight alone, when the latter is regarded as an independent factor.

Direct measurements of depth and length are involved in the relationships included in group 4 and designated 11, 13, 14, 15, and 16. All these coefficients are so low that the measurements are of little interest or significance in relation to the fatness of the carcass. In this connection, the relative values of depth of carcass (11), width through shoulders (2), and width through hams (8) are of special interest. Width through shoulders (+0.74±0.06) was distinctly superior to either of the others as an index of fatness, whereas width through hams showed only a slightly higher correlation than depth of carcass with percentage of fat in the edible portion of the carcass (+0.63±0.08 in comparison with +0.56±0.09).

SUMMARY

The method of measuring hog carcasses presented in this paper has been applied since 1929 to a large number of carcasses. The composition of 60 of these measured carcasses was determined by chemical analysis. Data were obtained from which it was possible to determine the relationships between certain measurements and the fat content of the edible portion of the carcasses.

The 60 measured and analyzed carcasses were those of hogs used in two cooperative experiments with the North Carolina Agricultural Experiment Station and three independent experiments. The experiments varied in nature and primary objectives. The hogs varied with respect to age, breed, sex, type, and initial weight, kind and quantity of feed, rate of gain, total gain, final weight, and market grade. Some were even fed to gain and then lose weight during the period of the experiment.

The thickness of back fat is generally regarded as a dependable, practical indication of the fatness of a hog carcass. It was measured on each carcass at five specific points, and the five measurements were averaged to obtain a value for average thickness of back fat. These average values were correlated with the percentages of fat, or ether extract, in the edible portion of the 60 carcasses. A correlation coefficient of +0.84 was obtained, the standard error of the coefficient being ±0.04. The equation derived for the regression line representing the relationship was percentage of fat in total edible portion of carcass = 22.45 + 0.691 × average thickness of back fat.

The accuracy sacrificed by employing this rapid, inexpensive method for estimating fatness is indicated by comparing its correlation coefficient with (1) the coefficient representing the relationship between the chemically determined fat content of the edible portion of the carcasses and that of the trimmed right hams and (2) the coefficient representing the relationship between the fat content of the carcasses and the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimmings. The three correlation coefficients are 0.84±0.04, +0.93±0.02, and 0.91±0.02, respectively. The coefficients of determination for the three methods of estimating fatness are 0.71, 0.86, and 0.83, respectively. The standard errors of the regression lines for the three methods are ±4.2, ±1.7, and ±3.3, respectively. The results show that, for representative animals, the average thickness of back fat is a hog-carcass characteristic of very definite value for estimating fatness of the edible portion of the carcass.

Separate consideration was given to 34 of the hogs, which were classed intermediate in type. A smaller standard error of the regression line for this group suggests that uniformity in type was a factor resulting in slightly greater accuracy in the estimation of fatness in the edible portion from the average thickness of back fat. However, the difference between the correlation coefficients for the two groups was not statistically significant.

A number of other measurements, also certain ratios, final feed-lot weight, and chilled-carcass weight were studied to determine their values as indices of the percentage of fat in the total edible portion of the hog carcass.

The 16 factors are classified into 4 groups. Group 1 includes those that involved direct measurement of the layer of external fat; group 2, those which appear as ratios; group 3, final feed-lot weight and chilled-carcass weight; and group 4, those appearing as direct measurements of depth and length.

Of the 6 highest correlation coefficients among the total of 16, the 2 highest values are in group 1, the next 2 in group 2, and the lowest 2 in group 3. Thickness of back fat at the seventh dorsal vertebra gave the highest coefficient (+0.77±0.05), width through shoulders being a close second. Weight per unit length of body plus leg and thickness of back fat at the seventh dorsal vertebra divided by depth of carcass (exclusive of back fat) gave coefficients only slightly lower than the latter.

Final feed-lot weight and chilled-carcass weight gave identical coefficients, too low to be of particular interest. Likewise, all group 4 factors showed little relation to the fatness of the carcass.

FACTORS AFFECTING GLADIOLUS IN STORAGE[1]

By J. I. LAURITZEN, *senior physiologist, Division of Sugar Plant Investigations*,[2] and R. C. WRIGHT, *physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*[3]

INTRODUCTION

The present knowledge regarding the proper storage conditions for corms of gladiolus (*Gladiolus* spp.) has been gained from experience and from isolated test trials under special conditions. The storage practice based on this knowledge consists in general of first curing the corms and then storing them in dry air at low temperatures. The curing consists in drying the corms and tops until the latter can be removed without injury to the corms. The most specific recommendations given regarding proper storage conditions are that the storage house should be dry and that its temperature should approximate 4.5° C. The necessity for keeping the corms dry has been further recognized in the practice of storing them in slatted shallow crates. A common practice is to store the corms in trays that slide like drawers in a rack several stories high, or (if the corms are small) on screen-wire shelves. Because of the low temperatures prevailing during most of the storage season (winter months) in the Northern States, common storage has been the usual practice. In recent years, because of the increased production of gladiolus in the southern part of the United States and the comparatively high temperatures of the storage season there, the practice of refrigerated storage has been initiated.

Soule,[4] working in Florida, found that more uniform germination occurred in corms stored for a time at cold-storage temperatures (probably 4.5° to 5° C.) than in corms not so stored (conditions not stated). He states that in corms stored at a temperature of 5° and a relative humidity of 70 percent there was no shriveling, no loss in weight, and no development of blue mold.

The investigations discussed in this paper were undertaken in response to a demand on the part of commercial dealers for more definite information regarding the effects of storage temperature and humidity on gladiolus.

A study was made of the effects of certain temperatures and humidities on dormancy, rooting, sprouting, loss in weight, and the development of penicillium rot in wounded and unwounded gladiolus corms during storage, and the subsequent germination, flower production, and yield of these corms in the field. Information was also sought regarding the relation of suberization and periderm formation in wounded areas to infection of gladiolus corms by *Penicillium gladioli* McC. and Thom, and the tolerance of corms to certain temperatures favorable for cork formation. The work was done at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C.

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[1] Received for publication Nov 1, 1933, issued, April 1934.

[2] Formerly pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

[3] The writers are under obligations to Dr. Freeman Weiss, of the Division of Fruit and Vegetable Crops and Diseases, who kindly arranged with the American Gladiolus Society to obtain from its members most of the corms used in these investigations and who assisted in the selection of the varieties and in diagnosing some of the diseases encountered, and to Lucia McCulloch, of the same Division, who assisted in diagnosing some of the diseased corms.

[4] SOULE, M. J. GLADIOLUS IN FLORIDA. Gladiolus Rev. 7: 103, 106, 133. 1930.

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MATERIAL

The following varieties[5] of gladiolus were used in storage experiments during 1 to 3 seasons (1927–28, 1928–29, and 1929–30): Alice Tiplady, Jenny Lind, Latinia, Lucette, Louise, Mrs. J. C. Bruggen, and White Wonder, 1 season; Maiden Blush and Virginia, 2 seasons; and Chicago White, 3 seasons. Chicago White, Latinia, Maiden Blush, and Virginia were used also during the storage season of 1930–31 in a study of the relation of wounding, suberization, and periderm formation to infection by *Penicillium gladioli*. These varieties were obtained from various parts of the country and were shipped to Washington, D.C., by express, late in the fall, after having been subjected to various conditions of curing and temporary storage at the respective points of origin. During the first season the corms of the different varieties before being stored at Arlington Experiment Farm were subjected to very different field conditions, climate, and handling during harvesting, curing, and shipment. After the first season's storage, however, the varieties used for more than one season were grown at the Arlington Experiment Farm under uniform conditions and were subjected to the same handling and curing methods. Thus the material, aside from differences in variety and in some instances in size of corms,[6] was fairly comparable. Before the storage experiments were begun, all lots of corms were thoroughly inspected and all abnormal specimens, such as those that had wounds or disease or were off-type, were discarded.

In the inoculation experiments *Penicillium gladioli* isolated from gladiolus was used as the inoculum.

CONTROL OF STORAGE CONDITIONS

The storage space used was of three types: (1) Insulated refrigerated rooms 8 feet wide, 14 feet long, and 11 feet high; (2)[7] galvanized-iron chambers 40 inches high, 42 inches long, and 35 inches wide; (3)[8] insulated chambers 2 feet high, 4 feet wide, and 3 feet deep.

The temperature of the insulated rooms was controlled either manually by refrigeration or automatically by a thermoelectric device. In the latter case the temperature of the room was reduced by refrigeration to slightly below that desired and then heated by means of electric heaters controlled by thermoregulators. The galvanized-iron chambers, 9 in all, were located in three of the rooms (3 in each room) in which the temperature was controlled thermoelectrically; the temperatures of these chambers were governed by those of the rooms. The temperatures of the insulated chambers were governed thermoelectrically and provided with air exchange. The humidity of the chambers and rooms was controlled by the use of water or calcium chloride in evaporation pans.

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[5] The original stock of the varieties used in these investigations was generously contributed by the following growers and dealers: Chicago White and Latinia varieties, by Vaughan Seed Store, Chicago, Ill.; Alice Tiplady, by Seabrook Nurseries, Seabrook, N.H ; Lucette, by Bill's Glad Farm, Canandaigua, N.Y.; White Wonder, by Ashville Flower Fields, Ashville, N.Y.; Maiden Blush, by Wales Road Gardens, Toledo, Ohio, Louise, by Deer Lodge Glad Farm, South Haven, Mich.; Mrs. J. C. Bruggen, by Hope Glad & Floral Gardens, Dallas, Tex.; Jenny Lind, by M. G. Ellis, Canby, Oreg.; and Virginia, by Briggs Floral Co., Encinitas, Calif.

[6] The following commercial grades were used as standards of size: No. 1, 1.5 inches in diameter and larger; no. 2, 1.25 to 1.5 inches; no. 3, 1 to 1.25 inches; no. 4, 0.75 to 1 inch; and no. 5, 0.5 to 0.75 inch. The sizes of the different varieties as they were received from the grower or dealer were as follows: Chicago White, Maiden Blush, and White Wonder, no. 1; Alice Tiplady and Louise, no. 2; Latinia and Lucette, no. 3; and Jenny Lind, no. 5.

[7] LAURITZEN, J. I., and WRIGHT, R. C. SOME CONDITIONS AFFECTING THE STORAGE OF PEPPERS. Jour. Agr. Research 41: 295–305, illus. 1930.

[8] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEETPOTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441–456, illus. 1923.

A different temperature was maintained in each of the 3 rooms containing the galvanized-iron storage chambers; the 3 temperatures were 0°, 4.5°, and 10° C. A different humidity was maintained in each of the 3 chambers in each room. An effort was made to maintain at 1 temperature 3 different humidities that should be comparable in evaporating power to 3 corresponding humidities maintained at each of the 2 other temperatures. In other words, the saturation deficits of the 3 humidities at 1 temperature approximated the saturation deficits of the 3 corresponding humidities at each of the 2 other temperatures. The temperature, saturation deficit, and humidity values sought are presented in table 1. The actual readings for the three seasons are given in subsequent tables. The insulated chambers and the galvanized-iron chambers were provided with psychrometers on which the air of a fan was directed. Each chamber contained glass windows so that readings could be made directly without disturbing the conditions within the chambers.

Table 1.—*Percentages of humidity at each of 3 temperatures corresponding to saturation deficits of equal value at the 3 temperatures*

Temperature (° C.)	Saturation deficit	Relative humidity	Temperature (° C.)	Saturation deficit	Relative humidity	Temperature (° C.)	Saturation deficit	Relative humidity
	Inches mercury	*Percent*		*Inches mercury*	*Percent*		*Inches mercury*	*Percent*
10	0.016	96	4.5	0.016	93.5	0	0.016	91
10	.037	89	4.5	.037	85	0	.037	79
10	.067	81	4.5	.067	73	0	.067	63

LOSS IN WEIGHT OF CORMS DURING STORAGE

It may be assumed that loss in weight of gladiolus corms in storage is due mainly to loss of water through evaporation and loss of carbon dioxide through respiration. The amount of carbon dioxide evolved is undoubtedly influenced by temperature but may also be influenced by wounding and possibly by the loss of moisture. In the absence of data relating to the interaction of these factors in the storage of gladiolus, it would be unsafe to assign to them a particular value. The loss of water is believed to be largely a function of the vapor pressure of corm tissue and of the air surrounding the corms, but doubtless it is influenced by wounding and the degree to which the wounded areas are healed by suberization and periderm formation. Proper maturing and curing previous to storage may also affect the loss of water. Such physical factors as the size and quantity of the corms and the amount of air movement play a part in modifying the loss in weight. The experiments reported herein were not designed to determine the value of these various factors but merely to determine the loss in weight of stored corms of a number of varieties under fairly uniform conditions of temperature and humidity (fig. 1). For a given variety during a given season, the methods of harvesting, curing, and wounding were uniform for all storage conditions, and corms of uniform size were used. As regards the different varieties, there were differences in the localities in which the corms originated and also differences in harvesting practice, wounding, curing, and the duration of storage. An effort was made to eliminate any obvious differences in wounding. Records of loss in weight under fairly comparable conditions of temperature and evaporating capacity of the air were made during three seasons for the Chicago White variety and during one season for seven other varieties (fig. 1).

The results show that loss in weight is correlated with evaporating power of the air, or saturation deficit. There were three exceptions in which the loss in weight was not in favor of the lower humidity:

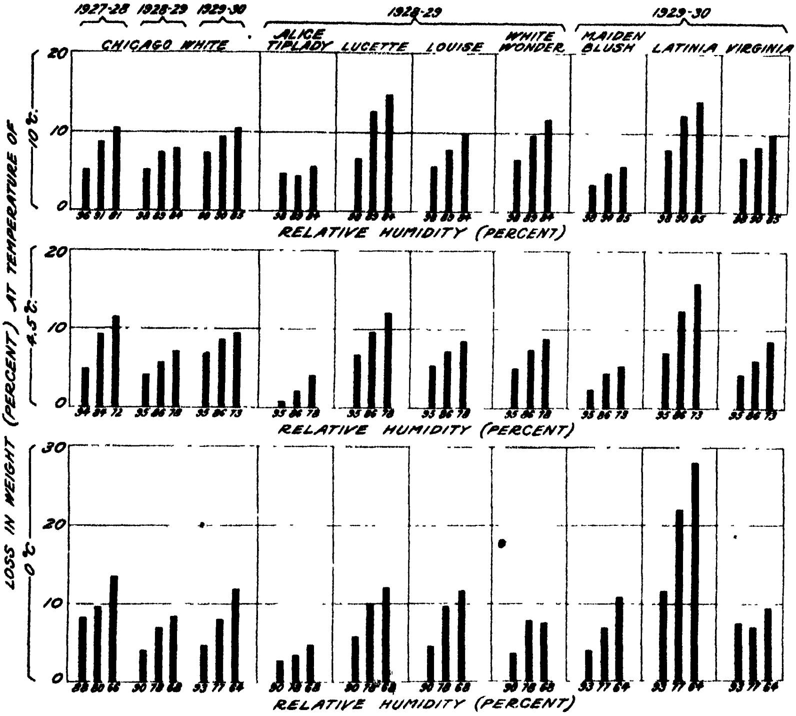

FIGURE 1.—Relation of temperature and humidity to percentage loss of weight in corms of gladiolus varieties during three seasons The duration of storage and the number of corms of each variety used under the various storage conditions were as follows During 1927–28, Chicago White, 47 days and 100 corms During 1928–29: Alice Tiplady, 83 days and 100 corms, Chicago White, 137 days and 100 corms, Lucette, 130 days and 85 corms, Louise, 73 days and 69 corms, and White Wonder, 80 days and 88 corms During 1929–30, Chicago White, 99 days and 85 corms; Maiden Blush, 99 days and 96 corms, Latinia, 96 days and 45 corms; and Virginia, 99 days and 39 corms.

(1) At 89 and 98 percent relative humidities at 10° C., in Alice Tiplady; (2) at 68 and 78 percent at 0°, in White Wonder; (3) at 93 and 77 percent at 0°, in Virginia. In most instances the percentage loss in weight at the three humidities was slightly greater at 0° C. than at 4.5°, and in some instances greater than at 10°, although it was greater more frequently at 10° than at 0°. The average loss at the three humidities at each temperature in all varieties was 8.2 percent at 0°, 7 percent at 4.5°, and 8.2 percent at 10°. Because of greater vegetative activity, such as rooting and sprouting, at 10° and 4.5° than at 0°, a higher rate of respiration would be expected at the higher temperatures, which would cause a greater loss in carbon dioxide and hence a greater loss in weight at humidities with the same saturation

deficit. The consistency with which a greater loss occurred at 0° than at 4.5° indicates that some factor other than humidity of the air or the loss of carbon dioxide through respiration functioned at 0° to increase the loss in weight. A possible explanation is suggested by the work of Artschwager and Starrett,[9] who found suberization and periderm formation occurring at 4.5° but not at 0°. It may be that a temperature of 0° inhibits thickening of the skin and consequently permits a greater loss of water, because of the immaturity or lack of continuity of the skin, than is possible at a higher temperature at which the thickening process can continue. Before definite conclusions can be drawn, however, more work must be done.

There was considerable difference in loss of weight among the different varieties, even in the same season under identical conditions of storage. Unfortunately these experiments were not designed to determine the relative susceptibility of the different varieties to loss of moisture. The corms varied in size, and the duration of storage was not the same (for various reasons) except in three varieties (Chicago White, Maiden Blush, and Virginia) during one season. The results emphasize the importance of making a comparative study in loss of weight in the different varieties.

ROOTING AND SPROUTING DURING STORAGE

Temperature and humidity affect not only the rooting and sprouting of gladiolus in storage but also the rest period or dormancy—a condition presumably resulting from a factor that inhibits some of the normal physiological processes, including germination and sprouting. However, no effort was made to isolate these latter effects. The present study was limited to the effects of certain storage conditions on sprouting and rooting during a period of 73 to 142 days (table 2). The data were obtained from the same experiments as were the data on loss of weight.

With the exception of 4 percent rooting and 2 percent sprouting in the Latinia variety after 99 days' storage at 0° C., the corms of all the varieties remained dormant at approximately 0°. There was some rooting at 4.5° in Chicago White during 3 storage seasons and in Lucette, White Wonder, and Virginia, but not in the other varieties. Except in one corm of Chicago White during 1 season (1929–30), there was no sprouting at 4.5° in any of the varieties. At 10° there was sprouting in Chicago White in 2 out of 3 seasons, and in all other varieties except Louise. There was rooting at 10° in all varieties except Louise. The storage period for the Louise variety was slightly shorter than that for any other variety and considerably shorter than that for some. Time may have been a limiting factor in this case. It is evident that temperature greatly affects both rooting and sprouting. In general, the higher the humidity of the storage chamber the higher was the percentage of rooting and sprouting. Hence as the humidity was lowered there was increased dormancy. There were certain exceptions, as in the small amount of rooting in Chicago White at 98 percent relative humidity at 10° during the season of 1928–29 and the small amount of sprouting under the same storage conditions during the season of 1929–30.

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[9] ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. Jour. Agr. Research 43: 353–364, illus. 1931.

TABLE 2.—*Relation of storage temperature and humidity to sprouting and rooting in different varieties* [a] *of gladiolus during 3 storage seasons*

Temperature (° C.) [b]	Relative humidity [c]	Condition of indicated variety during storage, season of—														
		1927-28 (Chicago White) [d]		1928-29 [e]												
				Alice Tiplady			Chicago White			Lucette			Louise	White Wonder		
		Dormant	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Dormant	Sprouting	Rooting
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10	96-98	0	100	1	76	99	71	5	29	9	57	87	100	5	94	95
10	89-91	100	0	100	0	0	8	4	92	24	22	74	100	0	0	100
10	81-85	100	0	100	0	0	49	0	51	29	0	71	100	100	0	0
4.5	94-95	0	100	100	0	0	51	0	49	85	0	15	100	17	0	83
4.5	84-86	100	0	100	0	0	84	0	16	90	0	10	100	100	0	0
4.5	72-78	100	0	100	0	0	90	0	10	91	0	9	100	100	0	0
0	88-93	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0
0	77-80	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0
0	64-68	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0

Temperature (° C.) [b]	Relative humidity [c]	Condition of indicated variety during storage, season of—											
		1929-30 [f]											
		Chicago White			Maiden Blush			Latinia			Virginia		
		Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10	96-98	51	1	48	0	85	100	0	98	100	0	74	100
10	89-91	81	4	13	7	93	0	100	0	0	44	5	56
10	81-85	91	1	8	8	92	0	100	0	0	77	3	21
4.5	94-95	0	0	100	100	0	0	100	0	0	0	0	100
4.5	84-86	99	1	0	100	0	0	100	0	0	100	0	0
4.5	72-78	86	0	14	100	0	0	100	0	0	100	0	0
0	88-93	100	0	0	100	0	0	96	0	4	100	0	0
0	77-80	100	0	0	100	0	0	98	2	0	100	0	0
0	64-68	100	0	0	100	0	0	100	0	0	100	0	0

[a] The number of corms of each variety used at each storage condition was as follows: Chicago White, in 1927-28, 100; in 1928-29, 100-124; and in 1929-30, 84-89; Alice Tiplady, 100, Lucette, 80-86; Louise, 69; White Wonder, 85-96; Maiden Blush, 92-98; Latinia, 45-46; and Virginia, 38-59.

[b] In order to simplify presentation of data, the temperatures sought were substituted for the actual temperatures obtained during each of the 3 seasons; however, the actual temperatures were close to those sought.

[c] The humidity readings represent the extreme humidities obtained at the particular storage condition during the 3 seasons. The actual temperature and humidity readings for the 3 seasons are given in tables 3, 4, and 5. This procedure is followed in later tables dealing with the same storage conditions. In the text only the temperatures sought are given.

[d] Storage period, 142 days; no sprouting.

[e] Storage period: Alice Tiplady, 83 days; Chicago White, 137 days; Lucette, 130 days; Louise, 73 days; and White Wonder, 80 days.

[f] Storage period, 96-99 days for all varieties.

FIELD TESTS ON GERMINATION, FLOWER PRODUCTION, AND YIELD OF CORMS

The success of any method of storing gladiolus is measured by the subsequent germination, production of plants and flowers, and yield of corms.

The corms of the Chicago White variety, stored during 3 seasons, those of the Lucette variety, stored for 1 season, and those of the Maiden Blush, Latinia, and Virginia varieties, stored for another season, were planted in the field, and a record was kept of germination, flower production, and yield of corms. The storage temperatures and humidities in all cases were fairly comparable.

TABLE 3.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in the Chicago White variety of gladiolus, 1927–28* [a]

Temperature during storage (° C)	Relative humidity during storage	Field germination: Corms planted [b]	Field germination: Plants yielded	Plants having indicated number of blooms (flower stalks): One	Two	Three	Four	Total plants having blooms	Yield of corms of indicated grade [c]: No. 1	No. 2	No. 3	No. 4	Total yield of corms
	Percent	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
5	96	75	95	20	51	26	3	100	145	35	11	4	195
3	91	75	99	22	55	20	3	96	137	55	12	0	204
7	81	75	97	16	57	21	6	96	132	31	9	0	172
6	94	75	99	23	53	21	3	95	164	59	12	16	251
5	84	76	100	11	72	17	0	92	201	34	11	3	249
5	72	75	100	22	60	17	1	96	193	43	9	0	245
0 2	88	75	97	31	41	24	1	93	179	47	12	5	243
0 3	80	75	97	26	60	14	0	89	150	60	19	0	229
0 3	66	75	77	41	49	10	0	71	128	59	17	5	209

[a] Storage period Dec 12, 1927, to May 2, 1928

[b] The corms were planted May 2, 1928, and harvested Aug 8 1928

[c] The corms were graded according to the following diameters No 1, 1 5 inches and larger, no. 2, 1 25 to 1 5 inches, no. 3, 1 to 1 25 inches, and no 4, 0 7 to 1 inch

TABLE 4.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in 2 varieties of gladiolus, 1928 29*

CHICAGO WHITE [a]

Temperature during storage (° C)	Relative humidity during storage	Field germination: Corms planted [b]	Field germination: Plants up June 1	Field germination: Plants up June 18	Field germination: Total plants	Plants having indicated number of blooms: One	Two	Three	Total plants having blooms	Yield of corms of indicated grade: No 1	No 2	No 3	No. 4	Total yield of corms
	Percent	*Number*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10 5	98	99	66	98	97	62	29	1	92	56	45	19	8	128
9 8	89	100	72	100	100	50	29	4	83	60	47	16	3	126
9 3	84	99	94	100	99	59	29	2	90	70	40	12	4	126
5 3	95	95	93	97	92	36	48	4	88	69	77	23	3	172
5	86	96	91	99	95	32	54	8	94	65	84	31	23	203
5	78	99	93	97	96	25	68	7	100	84	78	30	9	201
0 4	90	95	55	93	88	28	51	14	93	53	88	65	19	225
0 4	78	121	43	90	100	34	46	11	91	58	89	50	16	213
0 9	68	96	52	95	91	30	52	14	96	62	96	60	39	257

LUCETTE [c]

Temperature during storage (° C)	Relative humidity during storage	Field germination: Corms planted [b]	Field germination: Plants up June 1	Field germination: Plants up June 18	Field germination: Total plants	Plants having indicated number of blooms: One	Two	Three	Total plants having blooms	Yield of corms of indicated grade: No 1	No 2	No 3	No. 4	Total yield of corms
10.5	98	82	57	93	76	47	0	0	47	15	29	15	4	63
9.8	89	82	73	95	78	49	0	0	49	34	20	6	11	71
9.3	84	84	74	90	76	64	4	0	68	45	25	8	0	78
5 3	95	84	81	94	79	43	3	0	46	63	13	6	4	86
5	86	85	78	94	80	33	5	0	38	55	19	13	2	89
5	78	82	57	88	72	26	6	0	32	40	33	13	3	89
0 4	90	84	54	87	73	38	0	0	38	55	11	4	4	74
0.4	78	82	26	93	76	46	0	0	46	55	20	6	2	83
0.9	68	82	26	91	75	55	0	0	55	52	23	15	2	92

[a] Storage period, 137 days (Dec. 7, 1927, to Apr. 23, 1928).

[b] The corms were planted the first week in May and harvested Oct. 29, 1928.

[c] Storage period, 130 days (Dec. 14, 1927, to Apr. 23, 1928).

In soil of uniform character, the corms were planted 2 inches deep and 6½ inches apart, in 120-foot rows 30 inches apart. During the first season (1927–28) the corms of the Chicago White variety from each storage temperature were planted side by side in 3 rows, each row containing corms from the 3 storage humidities. During the second and third seasons, the corms from each condition of temperature and humidity were divided into two lots. One lot of each variety from each storage condition was planted in succession down and back up the rows, starting at one corner of the plot. This process was repeated for the second lot of each variety from the various storage conditions. The results shown in table 3 were obtained from 1 lot from each storage condition during 1927–28. The data in tables 4 and 5 represent averages of the 2 lots of each variety from each storage condition during the seasons 1928–29 and 1929–30, respectively.

TABLE 5.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in 4 varieties of gladiolus, 1929–30*[a]

CHICAGO WHITE

Temperature during storage (° C.)	Relative humidity during storage	Field germination			Plants having indicated number of blooms			Total plants having blooms	Yield of corms of indicated grade				Total yield of corms
		Corms planted[b]	Plants yielded		One	Two	Three		No. 1	No. 2	No. 3	No. 4	
	Percent	*Number*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10.0	98	84	81	96	84	10	0	94	57	21	21	7	109
10.0	90	84	83	99	86	5	0	91	67	32	12	2	113
10.0	85	84	82	98	81	1	0	82	60	26	19	5	110
5.5	95	42	42	100	71	14	0	85	71	24	12	0	107
5.0	86	64	64	100	84	5	0	89	77	14	11	6	108
4.8	73	84	84	100	86	8	1	95	82	19	7	2	110
0.2	93	60	60	100	73	18	0	91	32	70	18	13	133
0.2	77	72	72	100	82	18	0	100	22	60	51	14	147
0.8	61	80	78	98	77	13	0	90	37	53	36	14	140
						LATINIA							
10.0	98	44	44	100	39	54	0	93	4	48	75	43	170
10.0	90	46	45	98	64	36	0	100	24	48	15	48	165
10.0	85	46	44	96	64	29	0	93	30	37	33	61	161
5.5	95	40	40	100	52	48	0	100	27	90	37	23	177
5.0	86	44	41	100	41	57	0	98	27	84	41	32	184
4.8	73	44	44	100	48	36	0	84	9	45	41	57	152
0.2	93	44	44	100	41	54	0	95	5	36	82	100	223
0.2	77	44	38	86	29	55	0	84	11	48	48	63	170
0.8	64	46	38	83	26	45	0	71	15	48	24	52	139
						MAIDEN BLUSH							
10.0	98	92	89	97	72	24	0	96	60	48	29	27	164
10.0	90	96	95	99	68	18	0	86	85	33	19	5	142
10.0	85	90	89	99	62	22	0	84	73	89	13	19	144
5.5	95	94	93	99	70	29	0	99	82	34	8	16	140
5.0	86	96	95	99	71	28	0	99	58	49	30	24	161
4.8	73	92	92	100	65	29	0	94	69	51	21	11	152
0.2	93	62	59	95	61	39	0	100	52	53	32	29	166
0.2	77	92	89	97	63	28	0	91	43	39	42	29	153
0.8	64	96	94	98	63	32	0	95	98	23	22	8	151
						VIRGINIA							
10.0	98	28	27	96	56	29	15	100	14	50	54	7	125
10.0	90	34	34	100	44	47	9	100	3	35	82	50	170
10.0	85	38	33	87	67	33	0	100	13	42	45	47	147
5.5	95	10	10	100	40	50	10	100	40	30	40	60	170
5.0	86	34	34	100	53	35	9	97	76	47	15	15	153
4.8	73	38	37	97	43	32	19	94	0	21	58	71	150
0.2	93	38	38	100	19	39	42	100	5	13	103	163	284
0.2	77	36	35	97	51	23	26	100	8	17	92	83	200
0.8	64	38	38	100	24	42	29	95	8	26	87	97	218

[a] Storage period: Chicago White, Maiden Blush, and Virginia, 99 days (Dec. 18, 1929, to Mar. 27, 1930); Latinia, 96 days (Dec. 21, 1929, to Mar. 27, 1930).

[b] The corms were planted in April and harvested in early September 1930.

For all varieties, the percentages of corms yielding plants ranged from 77 to 100, but was generally rather high and uniform. The size of the corms used in planting differed somewhat in different varieties and this may have exerted some influence on the production of blooms (spikes) and the yield of corms, although the results are contradictory. The Lucette variety (table 4), with small-sized corms (grade 3), showed a low but rather uniform production of blooms and yield of corms from all conditions of storage. On the other hand, Latinia (table 5), with corms of the same size (grade 3), showed a high production of blooms and yield of corms from all conditions of storage, except the fairly low percentages of blooms from corms stored at 0.8°, 0.2°, and 4.8° C., and relative humidities of 64, 77, and 73 percent, respectively. With the exception of the Lucette variety, the percentages of plants producing blooms ranged from 71 to 100. The former percentage was obtained in the lots of Chicago White and Latinia that showed the smallest production of plants.

The foregoing data show little or no influence of storage conditions on germination (yield of plants) or on production of blooms.

If the total result from the three humidities at each storage temperature is considered, it will be found that in all varieties the percentage yield of corms was greater from corms stored at 0° C., than from those stored at 10°, although the difference was small in Lucette (table 4) and in Maiden Blush and Latinia (table 5). In Chicago White for each of the three seasons (tables 3, 4, and 5) and in Virginia (table 5), the yield of corms was greater from corms stored at each of the humidities at 0° than from those stored at the corresponding humidity at 10°. If the total result from the three humidities at each storage temperature is considered, it will be found that in Chicago White (except during one season) and in all other varieties the yield of corms was greater from corms stored at 4.5° than from those stored at 10°, although in Maiden Blush the difference was small. In Chicago White in one season (table 3) and in Latinia, Maiden Blush, and Virginia (table 5), the yield of corms increased as the storage temperatures were lowered from 10° to 0°. These data show that the storage temperature exerts some influence on the yield of corms in gladiolus. The subject should be investigated further.

DEVELOPMENT OF PENICILLIUM ROT AT DIFFERENT STORAGE TEMPERATURES AND HUMIDITIES

INFECTION IN UNWOUNDED CORMS

The rot under consideration was produced mainly by *Penicillium gladioli*, although in some instances the invasion of corms by other species of *Penicillium* and by other pathogenic organisms has been noted. The storage conditions in part of these experiments were the same as those already discussed, and the data on disease were obtained from the corms used in the rooting and sprouting experiments. Chicago White was stored for 3 years, and Alice Tiplady, Latinia, Louise, Lucette, Maiden Blush, Virginia, and White Wonder for one season. In the remaining experiments, the corms of Mrs. J. C. Bruggen, Jenny Lind, Maiden Blush, and Virginia were stored at 0° to 15.5° C., and at different humidities; in these experiments no effort was made to make the humidities comparable at different temperatures.

Infection by *Penicillium gladioli* at 10° C. was limited to one season, and to the Lucette, Chicago White, Louise, and White Wonder varieties (table 6). In most varieties infection was heavier at 0° and at 4.5° than at 10°. No infection occurred at any condition of storage in the Virginia variety. In some instances the percentage of infection increased with the rise in humidity at 0° and 4.5°. The dependence of infection upon wounding may account for the absence of a more definite relation of infection to temperature and humidity.

TABLE 6.—*Influence of storage temperature and humidity on infection by Penicillium gladioli and other organisms in different varieties of gladiolus during 3 seasons*

Temperature during storage (° C.)	Relative humidity during storage	Corms of designated variety infected by indicated organisms during season of—															
		1927–28,[a] Chicago White, by *P. gladioli*	1928–29[b]							1929–30[c]							
			Alice Tiplady, by *P. gladioli*	Chicago White, by *P. gladioli* after—		Lucette, by *P. gladioli* after—		Louise, by *P. gladioli*	White Wonder, by *P. gladioli*	Chicago White			Maiden Blush, by *P. gladioli*	Virginia, by *P. gladioli*	Latinia		
				83 days	137 days	76 days	130 days			Total	*P. gladioli*	Other organisms			Total	*P. gladioli*	Other organisms
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10	96–98	0	0	0	1	1.2	1.2	0	4.5	0	0	0	0	0	0	0	0
10	89–91	0	0	0	0	0	2.3	0	0	0	0	0	0	0	0	0	0
10	81–85	0	2	0	0	0	1.2	1.4	0	0	0	0	0	0	0	0	0
4.5	94–95	0	2	5.9	5.9	0	0	1.5	0	8.2	8.2	0	0	0	9.1	9.1	0
4.5	84–86	0	1	2	2	0	0	0	1.1	5.6	3.3	[d] 2.2	0	0	2.3	2.3	0
4.5	72–78	0	2	0	0	0	5.8	5.8	3.5	0	0	0	0	0	4.3	0	[e] 4.3
0	88–93	1.3	0	1	1	0	0	0	1.1	8.2	4.7	[f] 3.5	3.1	0	4.3	4.3	0
0	77–80	0	1	0.8	0.8	0	4.6	1.4	1.1	5.9	3.5	[g] 1.2	3.3	0	4.3	4.3	0
0	64–68	0	0	0	3	0	2.4	0	0	2.4	2.4	0	1	0	0	0	0

[a] Storage period, 142 days, number of corms stored at each humidity, 100 or 101

[b] Storage period: Alice Tiplady, 83 days; Chicago White, 83 and 137 days, Lucette, 76 and 130 days, Louise, 73 days, and White Wonder, 80 days Number of corms stored at each humidity Alice Tiplady, 93 to 101; Chicago White, 100 to 124; Lucette, 83 to 86; Louise, 69; White Wonder, 85 to 96

[c] Storage period, 96 to 99 days for all varieties. Number of corms stored at each humidity. Maiden Blush, 92 to 97; Chicago White, 84 to 89, Virginia, 38 to 46, Latinia, 44 to 46

[d] 1.1 percent, scab; 1.1 percent, unidentified decay.

[e] Unidentified decay.

[f] 1.2 percent, *Fusarium;* 2.3 percent, unidentified.

[g] *Fusarium.*

During the season of 1928–29 unwounded corms of the Mrs. J. C. Bruggen, Jenny Lind, Maiden Blush, and Virginia varieties of gladiolus were stored at temperatures of 0° to 15.6° C. and at different humidities (table 7). No infection occurred in the Mrs. J. C. Bruggen and Virginia varieties and only 3.9 percent in Jenny Lind at one temperature (15.6°). Rather heavy infection occurred in Maiden Blush at all temperatures, regardless of humidity. These results indicate that some factor other than humidity and temperature determined the initiation of decay under the various conditions of storage. The corms of the Maiden Blush variety used in this experiment were badly wounded and covered with spores of *Penicillium* when they arrived from Ohio. In preparing this lot, as well as all other lots, the infected and most seriously wounded corms were discarded. The other varieties showed little infection or wounding. The high percentage of infection in Maiden Blush is believed to have resulted from contamination of wounded corms with *Penicillium.*

TABLE 7.—*Influence of temperature and humidity on infection of 4 varieties of gladiolus by Penicillium gladioli, 1928–29* [a]

Temperature during storage (° C.)	Relative humidity during storage	Mrs. J. C. Bruggen		Jenny Lind		Maiden Blush			Virginia	
		Corms stored	Corms infected	Corms stored	Corms infected	Corms stored	Corms infected after—		Corms stored	Corms infected
							61 days	114 days		
	Percent	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Number*	*Percent*
15.6	89	90	0	77	3.9	75	0	29.3	60	0
12.5	64	90	0	79	0	72	5.5	19.4	60	0
10	84	90	0	79	0	76	12	12	70	0
4.5	81	90	0	69	0	74	10.8	10.8	60	0
4.6	66	90	0	79	0	75	13.3	22.7	60	0
2.2	76	90	0	80	0	75	4	21.3	60	0
0	85	91	0	80	0	75	16	58.7	60	0
0.5	73	89	0	78	0	75	0	40	60	0
0	62	90	0	80	0	75	9.3	36.0	60	0

[a] Storage period: Mrs. J. C. Bruggen, 69 days; Jenny Lind, 51 days; Maiden Blush, 61 and 114 days; Virginia, 51 days.

INFECTION IN WOUNDED CORMS

McCulloch and Thom[10] have shown that *Penicillium gladioli* is unable to penetrate the normal uninjured epidermis of gladiolus corms.

The Chicago White variety, during three seasons, and the Alice Tiplady, Lucette, Louise, White Wonder, Maiden Blush, Latinia, and Virginia during 1 season (table 8) were stored from 96 to 181 days at 9 combinations of temperature and relative humidity. In the experiments of 1927–28 and 1928–29 the corms were wounded by cutting a small wedge-shaped piece of tissue about 3 to 4 mm deep from the side of each corm; in the experiments of 1929–30 a small slice of tissue about 1 mm deep and about 10 mm in diameter was cut from the side of each corm. Wounded corms of the Mrs. J. C. Bruggen, Jenny Lind, and Virginia varieties were stored during the season of 1928–29 at the temperatures and humidities given in table 9. The method of wounding was the same as that used for other varieties in the experiments of 1927–28 and 1928–29.

Under the same conditions of storage, infection by *Penicillium gladioli* was much more consistently present in the wounded than in the unwounded corms. No infection occurred in wounded corms of the Virginia variety under the storage conditions shown in table 9, and very little after 99 days' storage at the temperatures and humidities shown in table 8. This variety, however, developed no infection in unwounded corms under either set of storage conditions.

There was no infection by *Penicillium gladioli* in wounded corms of any variety at any of the relative humidities at 10° C. (tables 8 and 9). The only infection that occurred at temperatures above 10° was 27 percent in the Jenny Lind variety at 15.6° (table 9). In the Lucette, Louise, and White Wonder varieties, infection was limited to a temperature of 0° (table 8). In the Mrs. J. C. Bruggen variety, infection was limited to 0° and 2.2° (table 9). In Chicago White (during three seasons), Alice Tiplady, Jenny Lind, Maiden Blush, Virginia (during one season), and Latinia, infection occurred at 0° and 4.5° (tables 8 and 9). Infection also occurred in the Jenny Lind variety at 0.5°, 2.2°, and 4° (table 9).

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[10] McCulloch, L., and Thom, C. A ROT OF GLADIOLUS CORMS CAUSED BY PENICILLIUM GLADIOLI L. MCC. AND THOM. Jour. Agr. Research 36: 217–224, illus. 1928.

TABLE 8.—*Influence of wounding on infection by Penicillium gladioli in different varieties of gladiolus at different temperatures and humidities during 3 seasons*

[Values for depth of decay during last two seasons represent averages]

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms [a] of designated variety during season of—									
		1927-28 [b]		1928-29 [c]							
		Chicago White		Alice Tiplady		Chicago White		Lucette		Louise	
		Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10	96-98	0	0	0	0	0	0	0	0	0	0
10	89-91	0	0	0	0	0	0	0	0	0	0
10	81-85	0	0	0	0	0	0	0	0	0	0
4.5	94-95	27	2.5-5	11	3	60	2	0	0	0	0
4.5	84-86	0	0	11	20	0	0	0	0	0	0
4.5	72-78	0	0	0	0	0	0	0	0	0	0
0	88-93	100	[d]19-25	100	9.3	80	3.4	33	2.5	80	1.5
0	77-80	100	[e]7-25	11	25	30	2	0	0	20	2.5
0	64-68	0	0	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms [a] of designated variety during season of—									
		1928-29 [c]		1929-30 [f]							
		White Wonder		Chicago White		Maiden Blush		Virginia		Latinia	
		Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay
		Percent	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10	96-98	0	0	0	0	0	0	0	0	0	0
10	89-91	0	0	0	0	0	0	0	0	0	0
10	81-85	0	0	0	0	0	0	0	0	0	0
4.5	94-95	0	0	80	3.6	90	5.0	0	0	10	1
4.5	84-86	0	0	30	6.2	60	2.4	20	3.0	0	0
4.5	72-78	0	0	0	0	0	0	0	0	0	0
0	88-93	50	4.8	100	3.3	100	4.4	30	1.3	30	.8
0	77-80	0	0	0	0	0	0	0	0	0	0
0	64-68	0	0	0	0	0	0	0	0	0	0

[a] The number of corms of each variety used under each condition of storage ranged from 9 to 12.

[b] Storage period, 181 days.

[c] Storage period: Alice Tiplady and Chicago White, 137 days; Lucette and White Wonder, 136 days; and Louise, 127 days.

[d] Decay penetrated to the center of the corm

[e] Decay mostly about 12 mm deep

[f] Storage period: Chicago White, Maiden Blush, and Virginia, 99 days; and Latinia, 96 days.

It is evident that the amount of moisture in the air is important in determining infection. With one exception, namely, the Jenny Lind variety at 4° C. and a relative humidity of 66 percent (table 9), no infection occurred at 4.5° or 0° at the lowest humidity employed and in many instances none occurred at the next higher humidity. In other words, nearly all the infection at each temperature occurred at the two highest humidities.

What are the factors limiting infection at 10° C. and at the lower humidities at 4.5° and 0°? In an effort to answer this question a study was made of the effects of temperature and humidity on suberization and periderm formation and their relation to infection by *Penicillium gladioli*.

TABLE 9.—*Influence of wounding on infection by Penicillium gladioli in 3 varieties of gladiolus at different temperatures and humidities, 1928–29*

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms of—					
		Mrs. J. C. Bruggen [a]		Jenny Lind [b]			Virginia [c]
		Corms infected	Average depth of decay	Corms infected	Depth of decay		Corms infected
					Average	Range	
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Mm*	*Percent*
	89	0	0	27	(d)	(d)	0
	64	0	0	0	0	0	0
	84	0	0	0	0	0	0
	81	0	0	9	10	10	0
	66	0	0	27	7.7	3-15	0
	76	8	3	64	7	3-18	0
	85	8	2	45	7	2-15	0
	73	0	0	18	7.5	10-15	0
	62	0	0	0	0	0	0

[a] Storage period, 122 days, 12 corms stored at each humidity.
[b] Storage period, 106 days, 11 corms stored at each humidity.
[c] Storage period, 104 days, 10 corms stored at each humidity.
[d] Completely decayed.

EFFECT OF SUBERIZATION AND PERIDERM FORMATION ON INFECTION BY PENICILLIUM GLADIOLI

Artschwager and Starrett made a histological study [11] of suberization and periderm formation in wounded gladiolus corms in the Maiden Blush variety stored at 24 combinations of temperature and relative humidity for 22 days. Nine of these combinations (3 humidities at each of the temperatures 0°, 4.5°, and 10° C.) were the same as those used in some of the experiments here reported (table 4). Data on suberization and periderm formation were taken daily for the first 10 days, then after 15 and 22 days. By the end of the first 10 days suberization had taken place at temperatures from 10° to 36.8°; by the end of 15 days it had developed at 4.5°. The rate of suberization was affected somewhat by temperature and humidity. Suberization developed at temperatures of 21.9° to 36.8° in 1 day, at 19.3° in 2 days, at 15.3° in 3 days, at 12.5° in 4 days, and at 10° in 5 days. The effect of lowering the humidities at 11.4° to 12.4° and at 28.3° to 28.8° was to retard suberization. At a temperature of 28.3° and a relative humidity of 61 percent, 3 days were required as compared with 1 day at the higher humidities at this temperature. In no instance was the humidity low enough at these temperatures to prevent suberization. Periderm formation occurred at temperatures above 15.3° within 10 days but not at temperatures below 15.3° in 22 days. It developed in 4 days at temperatures of 25.4°, 28°, 30.9°, and 36.8° with high humidity, and at 28° with humidities of 89 and 96 percent, but only after 5 days at 28° with humidities of 61, 72, and 79 percent. Five days also were required for its formation at 19.3° with high humidity.

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[11] ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. Jour. Agr. Research 43: 353-364, illus. 1931.

To rectify any misconception which may have arisen as a result of statements in the introduction and in footnote 7 of the paper here cited, Artschwager has submitted the following: "The impression given by us through the omission of a corrected statement, that our work consisted of more than a histological study of the materials furnished by J. I. Lauritzen is hereby corrected."

In another test, suberization and periderm formation occurred in wounded corms of the Chicago White, Maiden Blush, Latinia, and Virginia varieties in 79 days of storage at temperatures of 4.5° and 10° C., but not at 0°. Data were obtained from the same material as the data in table 10. No examinations of these lots were made at shorter intervals.

The foregoing results may serve to explain the presence or absence of infection in wounded corms (tables 8 and 9). Infection occurred in the absence of suberization and periderm formation (at 0° C.) or when these processes were greatly retarded (at 4.5°) and whenever the humidity was favorable to germination of spores and growth of *Penicillium gladioli* and other species of *Penicillium*. Infection was inhibited or prevented at temperatures and humidities favorable to rapid suberization and periderm formation. Temperature is more important than humidity in limiting or promoting these processes.

To test the effectiveness of a suberin and periderm layer in preventing infection, an experiment was run during the storage season of 1930-31 with the four gladiolus varieties Chicago White, Latinia, Maiden Blush, and Virginia. From 80 to 180 corms of each variety were wounded by cutting a slice from the side of each corm about 1 mm thick and about 1 cm in diameter. The corms were then stored for 10 days at 29° C. (relative humidity, 97 percent). Half of these corms were divided into nine equal lots and a lot of each variety was stored without further treatment at each of the nine combinations of temperature and relative humidity given in table 10. The other half of the corms were inoculated by applying spores of *Penicillium gladioli* to the wounded areas with a camel's-hair brush. They were then divided and stored in the same manner as the previous lot. A fresh quantity of corms of each variety was then similarly wounded. One half of these freshly wounded corms were stored without further treatment along with the cured corms; the other half were inoculated in the same manner as the cured corms, then divided and stored at the nine combinations of temperature and humidity. Table 10 shows the results obtained after 108 days of storage.

In the inoculated and uninoculated uncured wounded corms of all varieties infection occurred at 0° C. and 89 percent relative humidity, but none occurred at the two lower relative humidities (80 and 66 percent). At 4.5° infection in uncured corms was confined to the two highest humidities (94 and 85 percent), none occurring at the lower humidity (75 percent). In Chicago White and Latinia, infection occurred in both inoculated and uninoculated uncured corms at 4.5° at both 85 and 94 percent relative humidity. No infection occurred in uncured uninoculated Maiden Blush at 85 percent nor in uncured uninoculated Virginia at 94 percent relative humidity. It is evident that lowering the relative humidity at these temperatures limits infection in uncured, freshly wounded corms.

TABLE 10.—*Influence of fresh wounding and of wounding and curing on infection by Penicillium in 4 varieties of gladiolus after 108 days' storage at different temperatures and humidities*

CORMS WOUNDED AND CURED [a]

Temperature during storage (° C.)	Relative humidity during storage	Chicago White					Latinia			
		Inoculated			Not inoculated [b]		Inoculated [b]		Not inoculated [b]	
		Corms stored	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay
	Percent	*Number*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10.5	97	9	0	0	0	0	10	18	0	0
10.2	90	10	0	0	0	0	0	0	0	0
10.2	83	10	0	0	0	0	0	0	0	0
5.0	94	10	10	4	0	0	0	0	0	0
4.4	85	10	0	0	0	0	0	0	0	0
4.4	75	10	0	0	0	0	0	0	0	0
0.2	89	10	0	0	0	0	10	9	0	0
0.3	80	10	0	0	10	13	0	0	0	0
0.1	66	10	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Maiden Blush				Virginia					
		Inoculated [b]		Not inoculated [b]		Inoculated			Not inoculated		
		Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*
10.5	97	0	0	0	0	5	0	0	5	0	0
10.2	90	0	0	0	0	5	0	0	5	0	0
10.2	83	0	0	0	0	5	0	0	5	0	0
5.0	94	10	[c] 19	0	0	4	0	0	4	0	0
4.4	85	0	0	0	0	5	0	0	5	0	0
4.4	75	0	0	0	0	4	0	0	5	20	10
0.2	89	0	0	0	0	5	20	15	5	0	0
0.3	80	10	[c] 6	0	0	4	0	0	4	0	0
0.1	66	0	0	0	0	4	0	0	4	0	0

CORMS FRESHLY WOUNDED AND UNCURED

Temperature during storage (° C.)	Relative humidity during storage	Chicago White					Latinia			
		Inoculated			Not inoculated [b]		Inoculated [b]		Not inoculated [b]	
		Corms stored	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay
	Percent	*Number*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10.5	97	10	90	3	50	2.4	80	3.4	10	2
10.2	90	10	40	9	30	4	0	0	0	0
10.2	83	10	20	2.7	0	0	0	0	0	0
5.0	94	10	100	5.3	100	6	100	6.7	80	5.6
4.4	85	10	70	6.4	60	3.7	80	5.6	60	4.7
4.4	75	10	0	0	0	0	0	0	0	0
0.2	89	10	80	4.6	50	1.4	50	5.4	30	3.3
0.3	80	10	0	0	0	0	0	0	0	0
0.1	66	10	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Maiden Blush				Virginia					
		Inoculated [b]		Not inoculated [b]		Inoculated			Not inoculated		
		Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*
10.5	97	50	2.8	0	0	4	100	14	5	0	0
10.2	90	0	0	10	28	4	25	11	5	0	0
10.2	83	0	0	0	0	4	50	30	5	0	0
5.0	94	100	4.9	70	4.7	4	50	4	5	0	0
4.4	85	50	3.5	0	0	4	75	5	5	40	9
4.4	75	0	0	0	0	4	0	0	5	0	0
0.2	89	60	4.7	40	5.8	4	100	4	5	20	5
0.3	80	0	0	0	0	4	0	0	5	0	0
0.1	66	0	0	0	0	4	0	0	5	0	0

[a] The curing process consisted in storing the wounded corms for a preliminary period of 10 days at 29° C. (relative humidity, 97 percent).

[b] 10 corms were stored under each condition of treatment and storage.

[c] Infection was at the side of the wound.

In contrast to the results reported in tables 8 and 9, some infection occurred at 10° C. in uninoculated uncured corms in the Chicago White, Latinia, and Maiden Blush varieties. Infection was greater, however, in the inoculated uncured corms. It is possible that the presence of infection at 10° in the uninoculated corms may have resulted from increased contamination from inoculated corms. In most instances the decay was corked out[12] soon after it started. It should be stated that a *Penicillium* other than *P. gladioli* was also isolated from the infected corms.

Curing at a temperature of 29° C. and a relative humidity of 97 percent was effective in limiting infection in both inoculated and uninoculated corms. Very little infection occurred in any of the varieties at any of the storage conditions in either inoculated or uninoculated cured corms (table 10).

EFFECT OF CURING TEMPERATURE ON GERMINATION AND FLOWER PRODUCTION

In order to determine whether a curing process such as was employed in the foregoing experiment would injure the corms, 10 corms of the Maiden Blush variety were stored for 10 days at each of the temperatures and relative humidities given in table 11. These corms had been previously stored at 4.5° C. from November 18, 1929, to March 7, 1930. After 10 days in the various conditions of storage, the corms were planted (Mar. 17, 1930) on a bench in a greenhouse under uniform conditions.

These results show that a wide range of temperatures favorable to germination and production of flowers might be employed as a treatment preliminary to storage to insure healing in wounded areas. This curing process would not only have the advantage of protecting the corms against infection by *Penicillium gladioli*, but would also insure against loss of water incident to unhealed wounded areas and consequent shriveling.

Table 11.—Influence of 10 days' curing of gladiolus corms[a] *in the Maiden Blush variety at different temperatures and relative humidities, on sprouting and production of stalks and flowers*

[The corms had been stored previously at 4 5° C from Nov. 18, 1929, to Mar 17, 1930]

Temperature during curing (° C)	Relative humidity during curing	Sprouting: Date of first sprouts	Sprouting: Date of last sprouts	Production of plants and flowers: Date of first bloom	Production of plants and flowers: Plants produced	Production of plants and flowers: Flowers produced	Production of plants and flowers: Plants producing flowers
	Percent				*Number*	*Number*	*Percent*
37 0	87	Apr. 2	Apr. 10	June 18	23	18	78
31.0	93	Mar. 29	Apr. 5	June 13	20	17	85
28.5	97	do	Apr. 2	do	19	13	68
25.0	95	do	do	do	19	14	74
22 0	93	do	Apr 7	do	20	14	70
17.5	87	do	Apr. 10	June 16	21	14	66
15.5	91	Apr. 2	Apr. 7	June 20	24	20	83
12 5	93	Apr. 5	Apr. 10	do	23	13	57

[a] 10 corms were cured at each temperature; they were then planted in a greenhouse Mar 17, 1930.

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[12] A wound cork was formed beneath the decayed tissue.

SUMMARY AND CONCLUSIONS

At 0°, 4.5°, and 10° C. the loss in weight of corms of eight gladiolus varieties (Alice Tiplady, Chicago White, Latinia, Lucette, Louise, Maiden Blush, Virginia, and White Wonder) was found, with few exceptions, to increase at three relative humidities at each temperature as the saturation deficit increased.

Contrary to what was expected, the loss in weight at three humidities at 0° C. was greater in most instances than at three humidities with similar evaporating points at 4.5°.

With the exception of the Latinia variety (2 percent sprouting at 0.2° C. and 77 percent relative humidity and 4 percent rooting at 0.2° and 93 percent relative humidity), all corms remained dormant at approximately 0° C. during 73 to 142 days of storage. There was some rooting at 4.5° in the Chicago White variety during three seasons of storage. Rooting at this temperature was also observed in Lucette, Virginia, and White Wonder, but not in Alice Tiplady, Latinia, Louise, or Maiden Blush. Except in one corm of Chicago White during one season (1929-30), there was no sprouting in any of the varieties at 4.5°. At 10° there was sprouting in Chicago White in 2 out of 3 seasons of storage, and in all other varieties except Louise. There was rooting at 10° in all other varieties except Louise. This exception may have been due to the short storage period (73 days). In general, the higher the humidity of the storage chamber the higher the percentage of rooting and sprouting, at temperatures at which rooting and sprouting occurred.

The results of 3 seasons' work (1927-28, 1928-29, 1929-30) with Chicago White, of 1 season (1928-29) with Lucette, and of 1 season (1929-30) with Maiden Blush and Virginia, show that field germination was rather uniform in corms stored at three humidities at temperatures of 0°, 4.5°, and 10° C. The extremes of the range in percentage of corms producing plants were 77 and 100. Eliminating the two lowest percentages obtained (77 in Chicago White from corms stored at 0.3° and 66 percent relative humidity and 83 in Latinia at 0.8° and 64 percent relative humidity), the extremes of the range in percentages become 86 and 100. With the exception of the Lucette variety, the extremes of the range in the percentage of plants producing blooms were 71 and 100. The lowest production of blooms occurred in the lots of Chicago White and Latinia that produced the lowest percentage of plants. Otherwise, the production of flowers was rather uniform in all varieties regardless of the storage conditions. In contrast with other varieties, Lucette showed a low production of blooms. The small size of the corms (grade 3) may have been responsible for this low yield. However, Latinia, with corms of the same size, gave a high yield. The storage conditions employed in these experiments did not seem to affect the yield of plants or the production of blooms.

In all the varieties the total percentage yield of corms was greater from corms stored at 0° than from those stored at 10° C. The greater yields in Latinia, Lucette, and Maiden Blush were not so marked as in Chicago White and Virginia. In Chicago White (all three seasons) and in Virginia, the yields were greater from corms stored at the three humidities at 0° than from those stored at the corresponding humidities at 10°. In Chicago White in two seasons the yields were greater from corms stored at 4.5° than from those stored at 10°. In all other varieties, if the results from the three humidities at each temperature are considered, the yields were greater from corms stored at 4.5° than from those stored at 10° (difference in Maiden Blush, 1 percent). With the lowering of the storage temperature from 10° to 4.5° to 0°, there was a rising gradient in the yield of corms in Chicago White (in 1 out of 3 seasons), Latinia, Maiden Blush, and Virginia. These results indicate that storage temperature may have some influence on the yield of corms.

In sound unwounded corms stored at three humidities at each of the three temperatures 0°, 4.5°, and 10° C., there was very little infection by *Penicillium gladioli* in any of the varieties. Most of this infection occurred at 0° and 4.5°.

In one lot of Maiden Blush, which previous to storage had been subjected to rough handling and bore a superficial growth of *Penicillium*, there was a high percentage of infection under all the conditions of temperature and humidity at which the corms were stored. In corms that were wounded at the time they were stored, infection by *Penicillium* occurred at high humidities at 0° and 4.5°, but not at 10° C. during the three seasons 1927–28, 1928–29, and 1929–30, when no spores of *Penicillium gladioli* were introduced into the storage chambers. During the season of 1930–31, when spores of *Penicillium gladioli* were applied to some of the wounds some infection occurred in wounded, uncured corms stored at 10° in the absence of inoculation. At 0° and 4.5° it was generally true that the higher the humidity the higher was the percentage of infection. The lowest relative humidities (about 63 to 75 percent at 0° and 4.5°, respectively) practically eliminated infection, regardless of wounding and inoculation.

A suberin and periderm layer in wounded areas has been shown to be an effective barrier against infection by *Penicillium gladioli* and by other species of *Penicillium*. In corms of four varieties of gladiolus (Chicago White, Latinia, Maiden Blush, and Virginia), wounded and stored for 10 days at a temperature of 29° C. and a relative humidity of 97 percent and then stored for 108 days at 3 humidities at each of the 3 temperatures 0°, 4.5°, and 10°, very little infection occurred in any of the varieties under any of the storage conditions. This was true even of corms inoculated over the wounded surface at the end of the curing period before being stored. Corms wounded and stored without being cured showed the usual infection, whether inoculated or not, at the temperatures and humidities favorable for infection.

Data on germination and production of flowers in corms that had been stored for 10 days at 12.5°, 15.5°, 17.5°, 22°, 25°, 28.5°, 31°, and 37° C. and then planted in a greenhouse indicated that temperatures from 22° to 31° (favorable for suberization and periderm formation) might be employed to prevent infection and loss of moisture in unhealed wounded areas.

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DEVELOPMENTAL ANATOMY AND HOMOLOGIES IN WHEAT[1]

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INTRODUCTION

Embryo and seedling morphology and anatomy in various Gramineae, including wheat (*Triticum vulgare* Vill.[3]), have been studied intensively for more than 120 years. It would seem that homologies and other principal features should be definitely settled. However, divergent views presented even in some of the more recent papers dealing with the general subject (Boyd (*3*)[4], Avery (*1*), Howarth (*25*), Percival (*43*) emphasize the fact that observations have not been easily and certainly analyzed.

In 1897, Van Tieghem (*60*), in presenting a complete revision of his first interpretation published 25 years before, stated that "* * * malgré les nombreuses recherches dont il a fait l'objet, c'est encore aujourd'hui, dans l'histoire de ces plantes, l'un des sujets les plus controversés." This statement still applies.

Justification for presenting the present study lies in a hope that at least the observations submitted will help to clarify the situation without further confusing it.

REVIEW OF LITERATURE[5]

The literature of this subject is extensive. It has been well reviewed, however, by Van Tieghem (*59*), Bruns (*4*), Kennedy (*28*), and recently by Avery (*1*), and it seems unnecessary to present here more than a summary of the various conflicting views. Different interpretations of the grass embryo follow:

(1) The scutellum is the cotyledon, the coleoptile being considered the next succeeding leaf, or the first leaf of the plumule, and the epiblast, when present, either of no significance or an appendage of the axis, coleorhiza, or scutellum. This view has been held by Mirbel (*36*), Raspail (*46*), Demoor (*12*), Schacht (*51*), Heiden (*22*), Nobbe

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[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum* L ; but as *T vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form

[4] Reference is made by number (italic) to Literature Cited, p. 318.

[5] A more detailed review of the literature is filed in typewritten form in the library of the U S. Department of Agriculture, under the title "Review of Literature on the Morphology and Homology of the Grass Embryo", by M. A McCall.

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(*42*), Lermer and Holzner (surmise) (*31*), and Avery (*1*). Malpighi (*35*) is usually considered to have interpreted the scutellum as a seed leaf. It is difficult to tell, however, whether his phrase, "the leaf, which is called the flesh of the seed", refers to the scutellum or to the endosperm and seed coats. Mirbel (*37*) assumed that Gaertner's (*16*) ideas conformed to this view, but Gaertner's writings hardly justify this conclusion. Those supporting the view mentioned above consider that the interval of the axis between the attachment of the scutellum and the divergence of the coleoptile (the mesocotyl of maize) is internode.

(2) The scutellum is the cotyledon; the epiblast, when present, is a rudimentary cotyledon; and the coleoptile is the third leaf of the plant, or the first leaf of the plumule. This view has been held by Poiteau (*45*), A. L. de Jussieu, as reported by Mirbel (*37*), Mirbel (*39*), Turpin (*62*), Kratzmann (*30*), Warming (*63, footnote pp. 446–448*), Hackel (*19*), Bruns (*4*), Van Tieghem (*60*), Coulter (*10*), Weatherwax (*64*), and Percival (*43*). Opinion in this group varies as to the position of the epiblast. Some consider it opposite the scutellum at the same node. Others consider it as belonging to a second node between the scutellum and the coleoptile. This group also interprets the so-called mesocotyl as internode. Van Tieghem (*60*) credited Malpighi (*35*) with believing the epiblast to be a rudimentary cotyledon. There seems to be nothing, however, in Malpighi's (*35*) own statements or in the legends of his drawings to indicate such an opinion on his part. Mirbel (*37*) credits De Jussieu with originating this general theory, and it is on Mirbel's statement that he is placed in this group. Turpin (*62*), a contemporary of A. L. de Jussieu, Mirbel, and Poiteau, on the other hand states definitely that Poiteau (*45*) was the first to interpret the epiblast as a rudimentary cotyledon.

(3) The scutellum and the coleoptile together are the cotyledon, the epiblast, when present, being interpreted as an appendage of the axis, scutellum, or coleorhiza. Mirbel (*38*), Bernhardi (*2*), Schleiden (*52*), Hofmeister (*23*), Hanstein (*20*), Van Tieghem (*59*), Ekkert (*14*), Hegelmaier (*21*), Klebs (*29*), Schlickum (*53*), Čelakovský (*7*), Strasburger (*57*), Tannert (*58*), Sargant and Arber (*50*), Worsdell (*65*), Nishimura (*41*), Souèges (*56*), and Howarth (*25*) have held this view. The coleoptile is variously interpreted as ligular, bistipular, or an extension of the cotyledonary sheath. The axis interval between scutellum and coleoptile attachments is interpreted as nodal, as "middle" of the cotyledon, hence the mesocotyl of maize. Hegelmaier (*21*) credited Gaertner (*16*) with first suggesting this opinion, but beyond a possible implication in the legend of one of his illustrations, the writer has been unable to find evidence in Gaertner's writings to confirm this view.

(4) The scutellum and the coleoptile together are the cotyledon, and the epiblast when present is a rudimentary cotyledon. Kennedy (*28*), Cannon (*6*), and Bugnon (*5*) are exponents of this theory. The epiblast apparently is interpreted as a leaf opposite the scutellum at the same node.

(5) The view that the coleoptile is the cotyledon and the scutellum an outgrowth of the radicle or the axis, held by Richard (*47*), Adrien de Jussieu (*27*), Lestiboudois (*32*), Gris (*17*), Hofmeister (*24*), and Sachs (*49*), has not been championed in any important survey of the matter in recent years.

(6) Boyd (*3*) held that the scutellum is an evolutionary fusing of a sucker (as that of *Hedychium*) and a part of the cotyledonary ligule; that the epiblast is a fragment of the sheathing base of the cotyledon and of a part of the cotyledonary ligule; and that the coleoptile is equivalent to the first foliage leaf.

The difficulty of the problem is suggested by the fact that several of those who have studied it have held different views thereon at different times. Mirbel (*36, 38, 39*), Hofmeister (*23, 24*) and Van Tieghem (*59, 60*) are examples. Holzner (*31*) confessed his inability to arrive at any definite decision. While some one of the several interpretations may have held temporary advantage in general favor at one time or another, none has ever been universally accepted.

MATERIALS AND METHODS

Anatomical studies were made on mature wheat embryos and on seedlings, beginning with early germination. The material used was *Triticum vulgare*, varieties Turkey Red (C.I. no. 1558)[6] and Hard Federation (C.I. no. 4733). These varieties differ widely in morphological and physiological characteristics. Because of these contrasts it was hoped that anatomical features might be so emphasized in one or the other that observations and interpretation would be simplified. The two varieties do not differ essentially, however, in their general anatomy, and, while there are differences in degree, features evident in one are equally clear in the other. Both varieties have been described by Clark, Martin, and Ball (*9*).

Earlier studies [7] showed that, when grown under different temperature conditions, both Turkey and Hard Federation can be considerably modified morphologically. At temperatures of 16° C. and below, both develop the crown at a relatively low level, and all sections of the axis below the crown are comparatively short. At temperatures of 20° and above, the position of the crown is relatively high, and subcrown sections of the axis are correspondingly longer. This lengthening extends even to portions of the axis that normally show little elongation, as for instance the section between scutellum and coleoptile divergences. This is particularly pronounced in Hard Federation plants.

It is apparent that many of the difficulties in interpreting the morphology of the wheat embryo and of the structures arising from it are due to the normally compressed and restricted longitudinal extent of the embryo axis below the plumule and of those sections of the plant axis formed directly from this part of the embryo axis. Any lengthening or extension in this region during germination therefore should be helpful. To obtain as much variation in this respect as possible, different lots of material of the two varieties chosen for study were grown in a controlled-temperature greenhouse in sections held at approximately 12°, 16°, 20°, and 24° C., respectively. A smaller quantity of material also was grown in controlled-temperature soil tanks.

Material for detailed anatomical studies was harvested at regular intervals from early germination, fixed in formal-acetic-alcohol, and embedded in paraffin by the usual methods. Sections were cut at 12μ to 18μ, depending on age. It was necessary to soften some of the oldest material in equal parts of concentrated hydrofluoric acid and 70 percent alcohol for 24 hours before embedding it, in order to cut satisfactory sections. Flemming's triple stain was used.

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[6] C.I. no. refers to accession number of the Division of Cereal Crops and Diseases.

[7] TAYLOR, J. W., and MCCALL, M. A. THE INFLUENCE OF TEMPERATURE AND OTHER FACTORS ON THE MORPHOLOGY OF WHEAT SEEDLINGS. [Unpublished manuscript.]

Drawings were prepared by taking a 5- by 7-inch photomicrograph of any desired section, making from this an ordinary bromide enlargement (about 200 diameters), inking-in the enlargement with waterproof india ink, and bleaching out the photograph with a potassium cyanide-iodine solution. This left a black and white line drawing, which, after being washed and dried, was reduced by photographing. In inking-in the enlargements all doubtful features were confirmed from the original section under the microscope. A similar technic has been described by Naylor (*40*).

The method used by the writer gives illustrations absolutely correct in proportion, and, if carefully checked, as correct in general detail as can be expected from any system involving the human factor (drawing ability, interpretation, etc.). The drawings can be made large enough so that, when they are reduced, irregularities practically disappear. A very decided advantage of the method lies in the fact that the inking-in may be dropped at any stage and taken up again even for a very short interval with no sacrifice in accuracy or necessity of setting up or of constantly readjusting delicate apparatus. Enlargement also may bring out details overlooked when the section was examined under the microscope.

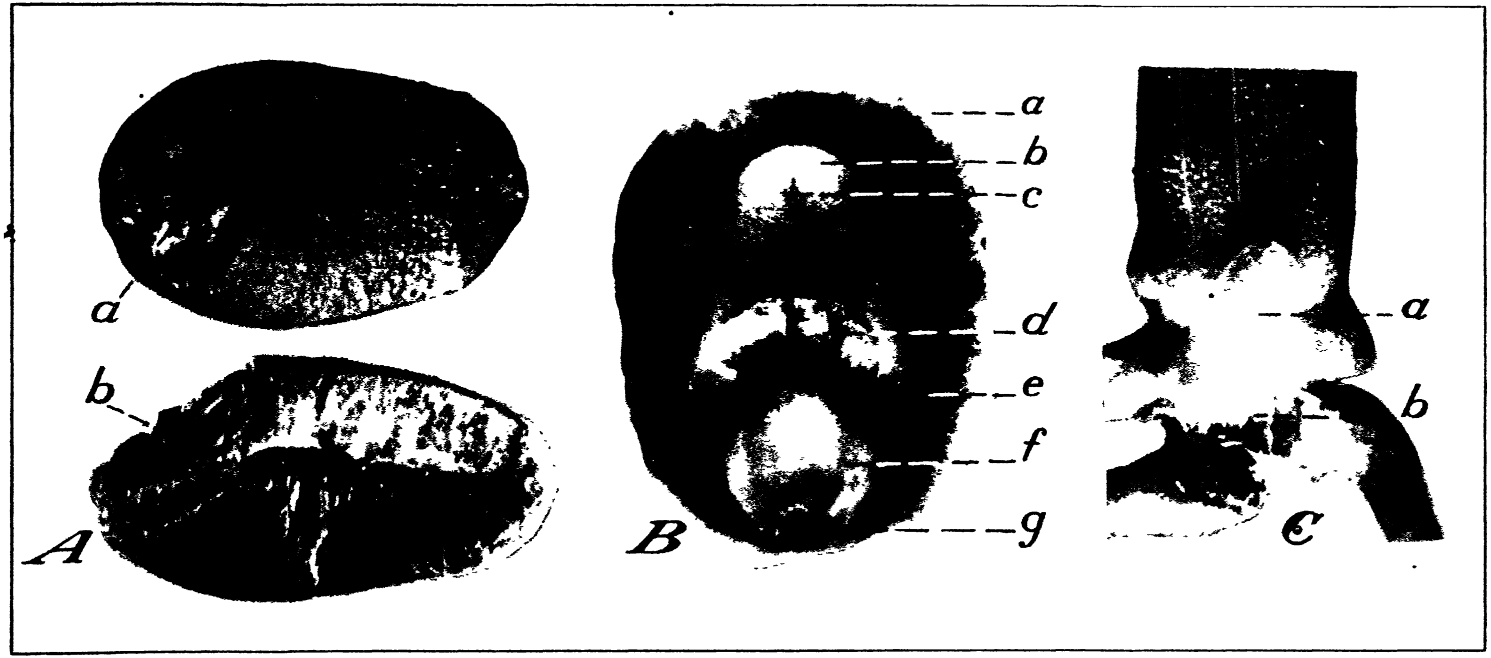
In making drawings by this technic, care must be exercised in using the cyanide-iodine solution, for if it is too strong, the ink as well as the photographic image is removed. The cyanide also is a deadly poison and must be thoroughly removed by careful washing.

OBSERVATIONS

GENERAL MORPHOLOGY

Excellent descriptions of the wheat embryo and of its developmental morphology during germination and early seedling stages are available from several authors. Van Tieghem (*59*), Percival (*43*), and Avery (*1*) all give good accounts. Features are described here only as necessary for identification and later discussion.

The embryo lies in the basal portion of the wheat grain on the dorsal side between the endosperm and the seed coat. Its position is shown in Plate 1, *A*, *a*, *b*. Externally the embryo consists of (1) an axis with a terminal plumule completely enclosed in a tubular cone-shaped sheath, the coleoptile, which is entirely closed except for a vent or slitlike pore in its front face slightly below the tip; (2) a terminal primary root enclosed within a cone-shaped root sheath, the coleorhiza; (3) an oval, fleshy structure, the scutellum (*16*), attached to one side of the axis and making up most of the bulk of the embryo; and (4), opposite to the scutellum, a small scalelike structure, the epiblast (*47*). In all well-developed embryos there is a pair of opposite lateral lobes on the axis in the general region which bears the epiblast. These are the outward evidence of the lateral seminal roots mentioned hereafter. As the embryo lies in the seed, the scutellum is closely appressed to the endosperm, the axis side of the embryo facing the seed coat. A view of the embryo from its front or axis face is shown in plate 1, *B*.



A *a*, Dorsal view; and *b*, sagittal section through a wheat grain, showing position of embryo × 10 *B*, Face or axis view of a wheat embryo *a*, Scutellum, *b*, coleoptile, *c*, vent of coleoptile, *d*, epiblast, *e*, lobe caused by lateral root, *f*, coleorhiza, *g*, suspensor × 30 *C*, Enlarged view of bud in a crown-leaf axil, showing the prophyll, the leaf torn away to expose the bud *a*, The bud with prophyll, *b*, the leaf base where the leaf has been torn away × 8

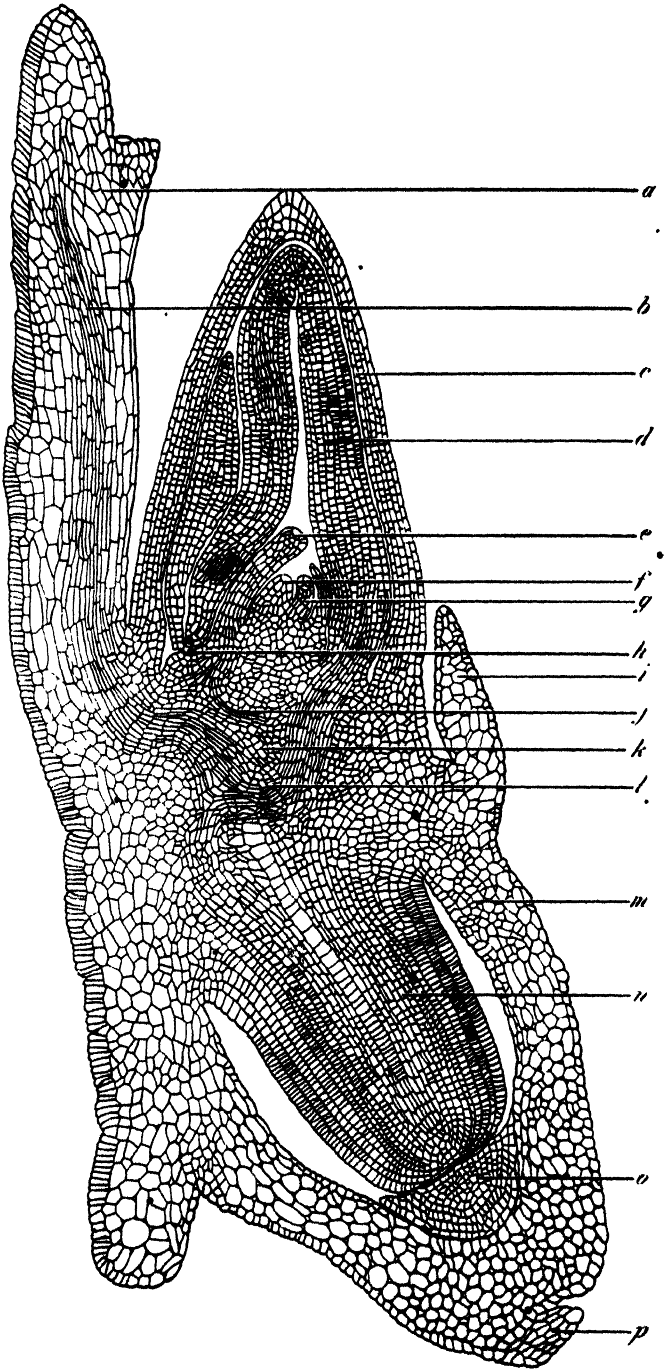


FIGURE 1.-- Median sagittal section of a wheat embryo (Turkey variety): *a*, Scutellum; *b*, scutellum trace; *c*, coleoptile; *d*, first foliage leaf; *e*, second foliage leaf; *f*, third foliage leaf, *g*, growing point; *h*, coleoptile axillary bud primordium; *i*, epiblast; *j*, procambium cross-axis plate, second node; *k*, first internode; *l*, first node, transition from root to stem; *m*, coleorhiza; *n*, primary root; *o*, root cap; *p*, remains of suspensor. × 80.

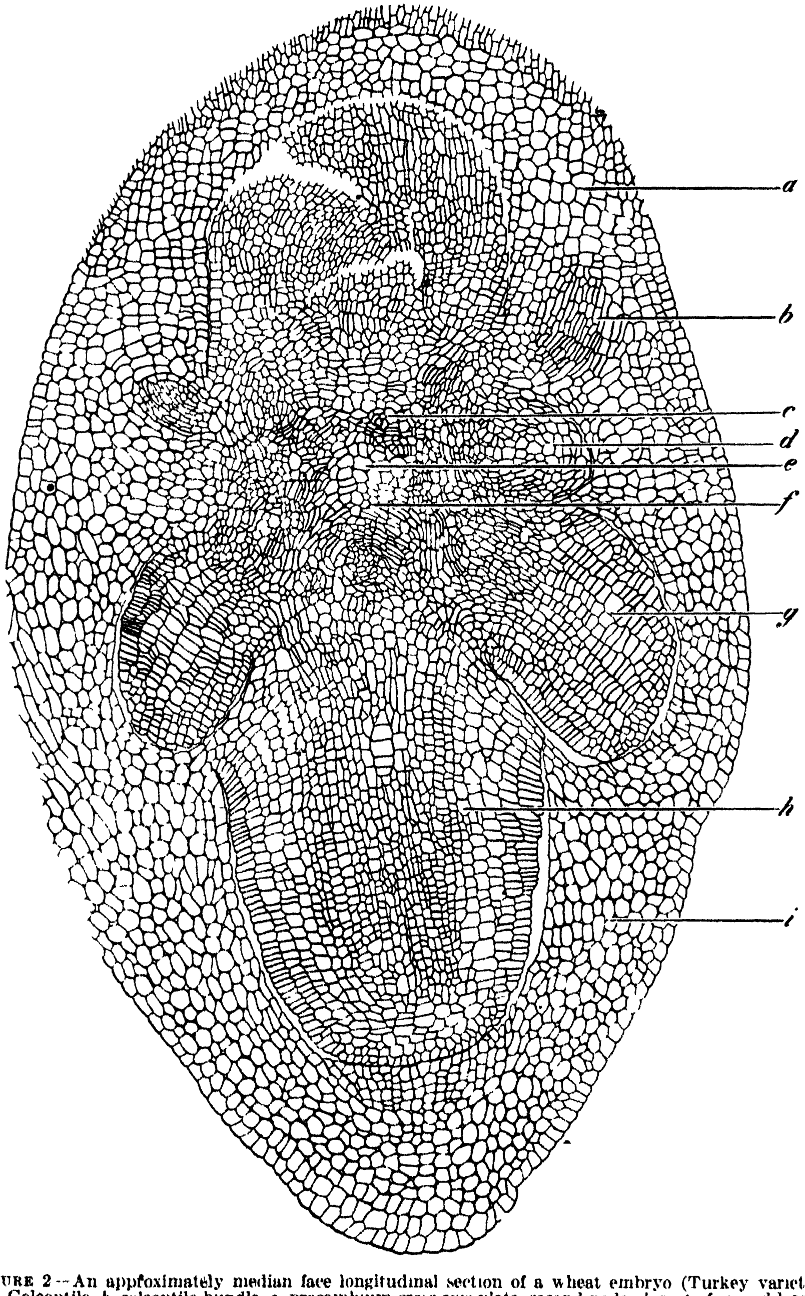


FIGURE 2 --An appfoximately median face longitudinal section of a wheat embryo (Turkey variety) *a*, Coleoptile, *b*, coleoptile bundle, *c*, procambium cross-axis plate, second node, *d*, root of second lateral pair; *e*, first internode; *f*, procambium cross-axis plate, first node; *g*, root of first lateral pair, *h*, primary root; *i*, coleorhiza. × 80.

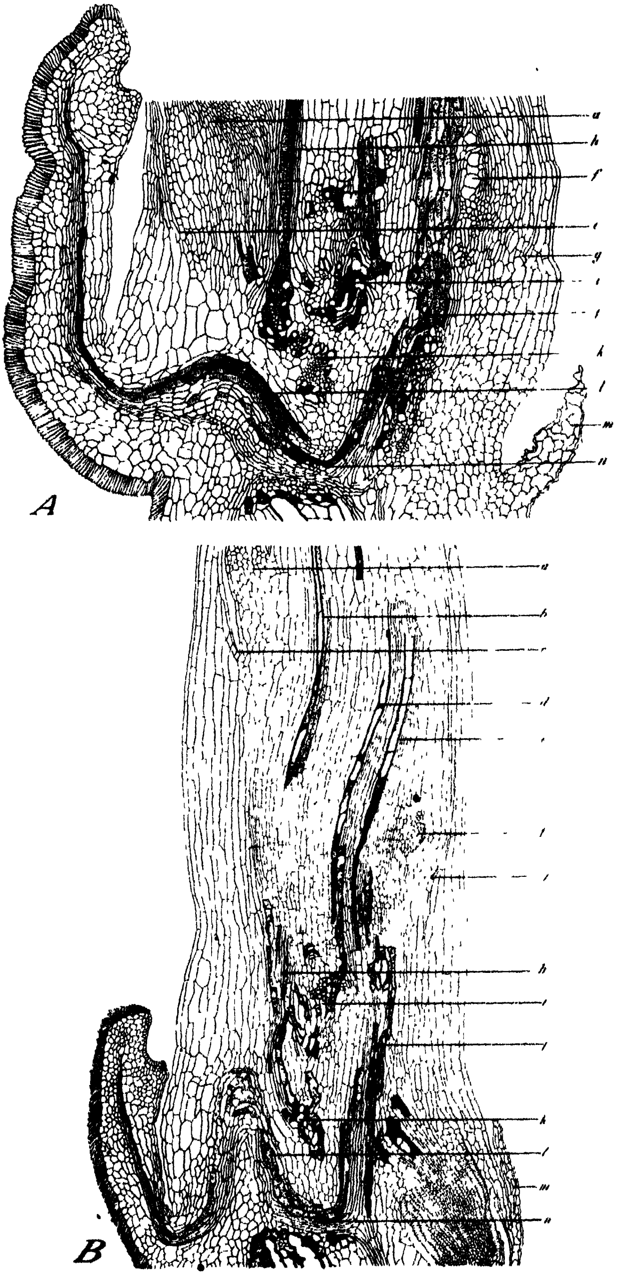


FIGURE 3.—Approximately median sagittal sections through the lower subcrown axis of Hard Federation wheat seedlings: *A*, A seedling with approximately normal elongation in this region, grown at 16° C.: *a*, Coleoptile axillary bud; *c*, posterior coleoptile axis attachment; *f*, tangential vascular connections of coleoptile axillary root; *g*, anterior coleoptile axis attachment; *h*, bundle *2-m* (bundle designations given in figs. 5 to 9, inclusive); *i*, vascular anastomosing region of third node; *j*, bundle *1-m*; *k*, vascular plate of second node; *l*, scutellum trace; *m*, epiblast; *n*, first node, transition of root to stem. × 45. *B*, A seedling with more than normal elongation in this region, grown at 20° C.: *a*, Coleoptile axillary bud; *b*, bundle *1-3l*; *c*, posterior coleoptile axis attachment; *d*, bundle *2-1r*; *e*, bundle *1-1l*; *f*, coleoptile axillary-root primordium; *g*, anterior coleoptile axis attachment; *h*, bundle *2-m*; *i*, vascular anastomosing region of third node; *j*, bundle *1-m*; *k*, vascular plate of second node; *l*, scutellum trace; *m*, epiblast; *n*, first node × 30. This section is at a slight angle to the right and in the upper part does not show bundles *1-m* and *2-m*.

In the leaf axil at each node there is a bud primordium, similar to that in the axil of the coleoptile. These bud primordia develop into secondary axes or tillers, each similar to the primary axis except that its orientation in respect to phyllotaxy, etc., is at 90° to that of the primary axis.

As already noted, during the earlier development of the wheat plant the culm internodes do not elongate. The closely grouped leaves from the crown nodes of the primary axis and of the tillers produce a tuft or rosette, characteristic of the so-called tillering stage in the life history of most grasses. During this stage bud and tiller formation are most active. The present study is not concerned with structures beyond this stage. A good description of developmental morphology for this and later stages is given by Percival (*43*).

The crown is included in this survey only because of certain positional and vascular relationships of leaves, roots, and nodes, and because of the axillary buds and their prophylls. The prophyll is a characteristic sheathing structure enclosing each axillary bud, and generally interpreted as its first leaf. It is entirely devoid of blade. A bud with its young prophyll is shown in plate 1, *C*. The similarity in appearance of the prophyll and the coleoptile (pl. 1, *B*) is evident. The anatomical features of crown and prophyll that bear on this study will be discussed later.

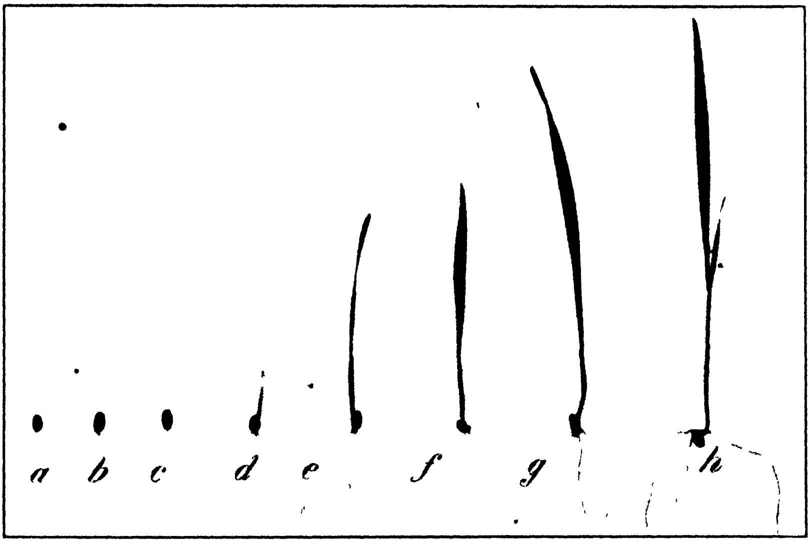


FIGURE 4. Relative development of Hard Federation wheat germinated at 16° C., by 2-day intervals to 14 days: *a*, Dry seed, *b*, 2 days, *c*, 4 days, *d*, 6 days, *e*, 8 days; *f*, 10 days, *g*, 12 days, *h*, 14 days. Two fifths natural size

By the time the first crown node can be identified most of the subcrown structures utilized in the identification of embryo homologies are evident. The exceptions are the adventitious roots which appear just above the divergence of the coleoptile some time after the first crown node has reached its permanent position. There may be only 1 of these roots, or 2, or more rarely 3. Their position varies almost entirely around the axis. The primordia of these roots are not present in the embryo, and therefore they cannot be properly classed as seminal roots, even though their position so close to the seed and far below the crown might suggest such a designation. As a rule, the growth of these roots is relatively slow, and their primordia may be present for some time before they are externally discernible, which may not be for a month or more after germination begins. In many cases they merely start growth and then make little subsequent development. The primordia of two roots originating just above the attachment of the coleoptile are shown in cross section in figure 5, *A*.

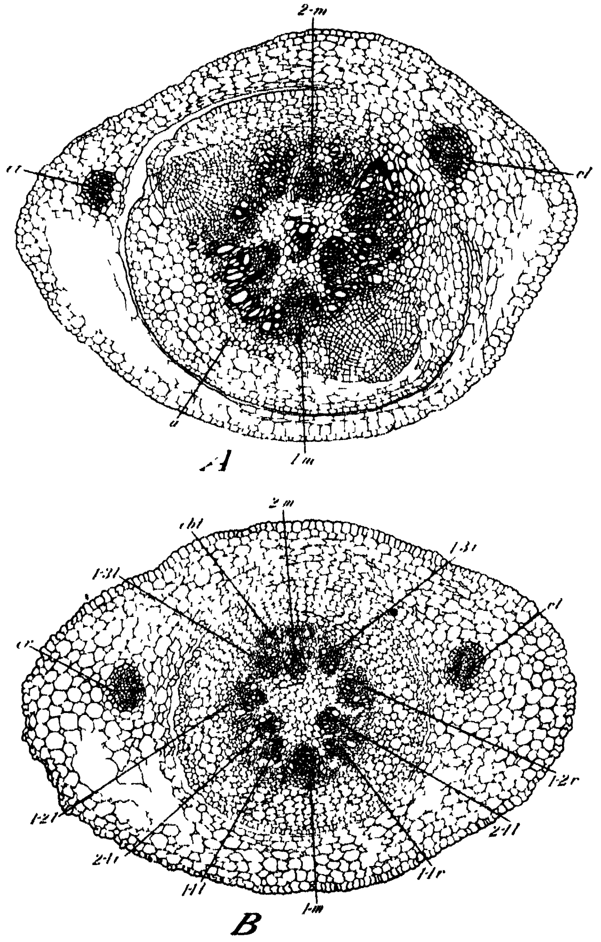


FIGURE 5.—Cross section of the stem axis of wheat seedlings just below complete coleoptile divergence: *A*, a 22-day-old Hard Federation wheat plant grown at 20° C., showing root primordia: *a*, Endodermis, *cl* and *cr*, coleoptile bundles; *1-m*, median bundle of first foliage leaf; *2-m*, median bundle of second foliage leaf. × 44. *B*, a 14-day-old Turkey wheat plant. *cl* and *cr*, Coleoptile bundles; *cbl*, vascular connections of the coleoptile axillary bud, *1-m*, median bundle of first foliage leaf; *1-1l*, *1-2l*, *1-3l*, *1-1r*, *1-2r*, *1-3r*, lateral bundles of first foliage leaf; *2-m*, median bundle of second foliage leaf; *2-1l*, *2-1r*, lateral bundles of second foliage leaf. × 44

There may be a maximum of 6 seminal roots in the wheat embryo. However, the primordia of all these are not present in every embryo. In many instances the primordium of a root on the front face of the axis is absent. This was true of the seedling from which figures 5, *B*, to 9, *B*, were drawn. In some cases there may be a primordium for only one of the second pair of lateral roots (fig. 2). Assuming that there are 3 roots, which may develop above the point of attachment of the coleoptile, a maximum of 9 roots may develop under optimum conditions in the subcrown region of the wheat plant. This, of course, does not apply in cases in which there are several nodes and internodes between the seed and the crown. This latter condition, described by Percival (*43*), is not unusual, but it is not typical. Nor does the usual rule apply when adventitious roots develop on the subcrown internode. Such a condition is rather rare, however, and when it does occur, the roots usually develop in close proximity to a node. Such a root, growing from the subcrown internode just under the first crown node, is shown in figure 10, *A*. The number of subcrown roots that actually

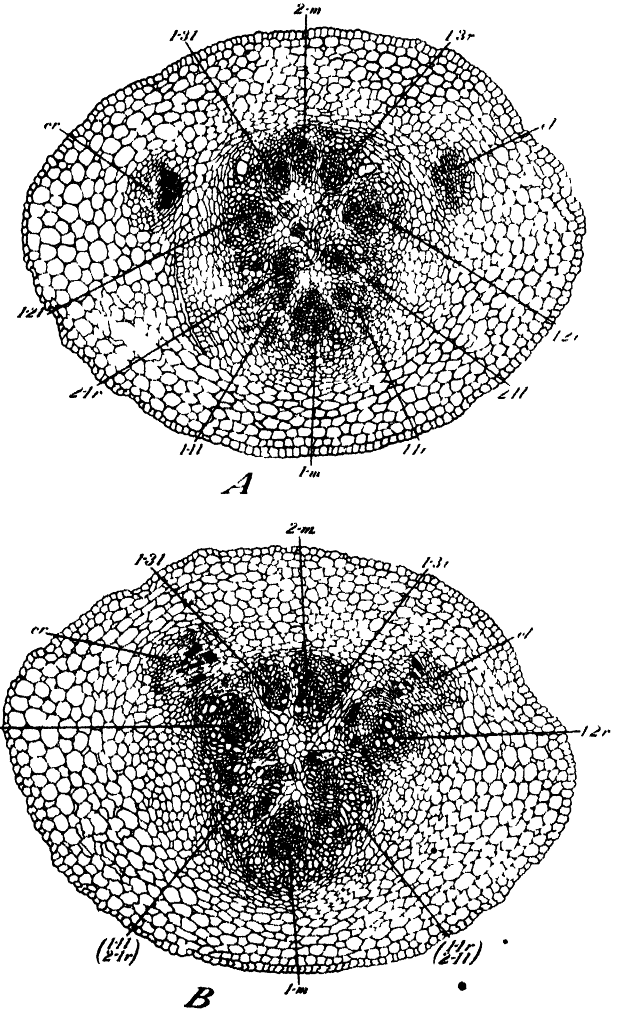


FIGURE 6.—Cross section of the stem axis (same plant as in fig. 5, *B*): *A*, cross-axis vascular connections of region interpreted as third node, just below coleoptile divergence; 0.42 mm lower than figure 5, *B*. × 44. Designations as in figure 5, *B*. *B*, 0.16 mm below figure 6, *A*, in region interpreted as internode. Designations as in figure 5, *B*. × 44.

appear is variable, usually ranging from 3 up to the potential maximum of 9. The relation of vascular anatomy to this variability in the number of developing roots will be discussed later.

An enlarged view of the basal region of a seedling wheat plant, showing the different structures discussed above and their positional relationships, is shown in plate 2, *C*. The portion of the plant axis below the point of divergence of the coleoptile represents the direct maturing of embryonic structures. Primary attention will be directed to vascular and positional relationships in this region because of their

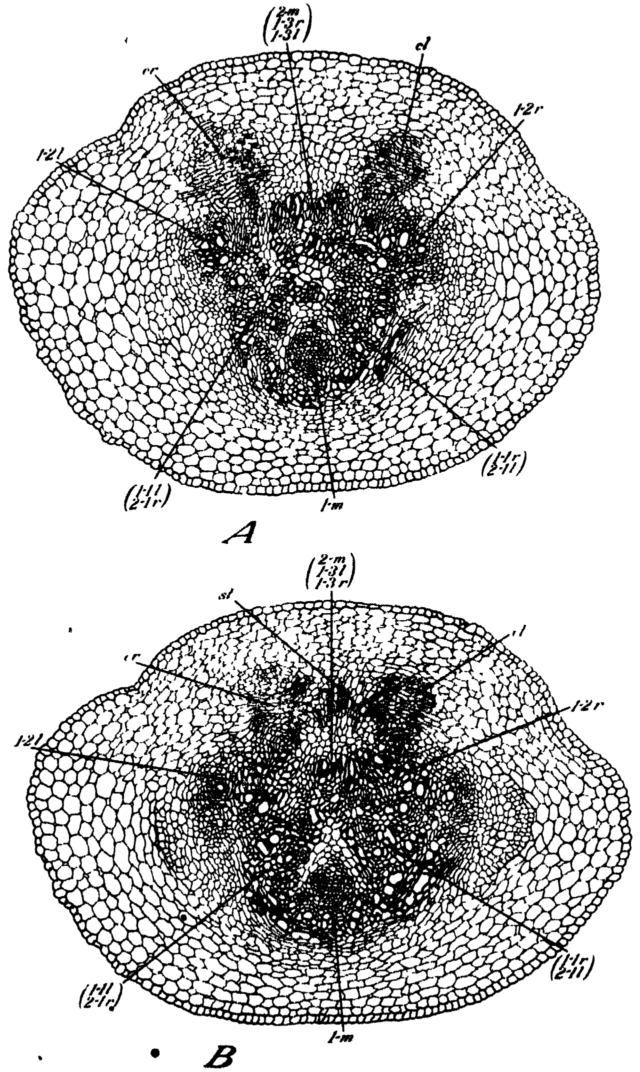
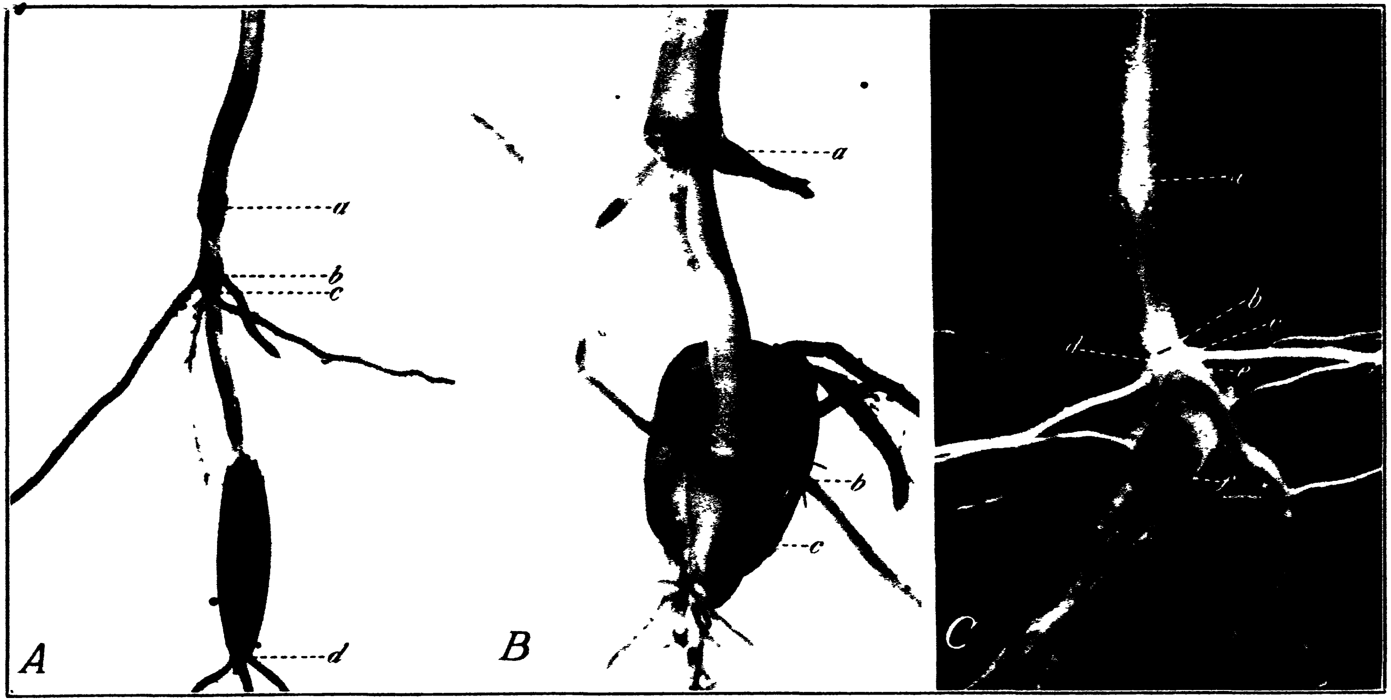


FIGURE 7 Cross section of the axis (same plant as fig. 5, *B*): *A*, 0.16 mm below figure 6, *B*, in region interpreted as internode, showing divergence of coleoptile bundles to back of axis, vascular connection between coleoptile bundles and stelar bundles, top of divergence of second pair of lateral seminal roots, and less distinct differentiation of stelar bundles. Designations as in figure 5, *B*. × 44. *B*, 0.1 mm below figure 7, *A*, in upper part of second node, showing junction of coleoptile bundles with scutellum trace, connection of coleoptile bundles with stelar bundles, vascular plate of second node, and divergences of second pair of lateral seminal roots and their vascular connections. *sl*, Scutellum trace, other designations as in figure 5, *B*. × 44



ENLARGED VIEWS OF THE SUBCROWN REGION OF 14-DAY-OLD SEEDLINGS OF OATS CORN, AND WHEAT SHOWING COMPARABLE MORPHOLOGICAL FEATURES

A, Iogold oat seedling, grown at 16° C *a*, Fourth node, crown node, *b*, third node, coleoptile divergence, *c*, second node, scutellum-trace divergence, *d*, first node, transition from root to stem *B*, Garrick corn seedling, grown at 18° C *a*, Third node, coleoptile divergence, crown node, *b*, second node, scutellum-trace divergence; *c*, first node, transition from root to stem. *C*, Turkey wheat seedling, grown at 16° C *a*, Fourth node, crown node, *b*, third node, coleoptile divergence, *c*, second node, scutellum-trace divergence, *d*, epiblast, *e*, first node, transition from root to stem, *f*, primary root The upper black line on the face of the seedling marks coleoptile divergence and the third node, and the lower black line the transition from root to stem or the first node. This region of the axis develops by the direct maturing of embryo tissues All × 2½.

bearing on embryo interpretation. The position of this region is defined also in plate 2, *C*.

DETAILED ANATOMY

A median sagittal section of an embryo from a mature wheat seed is shown in figure 1. The positional relations of axis, scutellum, radicle, plumule, and epiblast are plain. This section bisects longitudinally certain vascular-bundle primordia, the long narrow procambial cells of which (fig. 1, *l*) can be differentiated from surrounding cortical and pith parenchyma. The procambium, being meristematic, takes and retains the safranin stain more than parenchyma, a fact

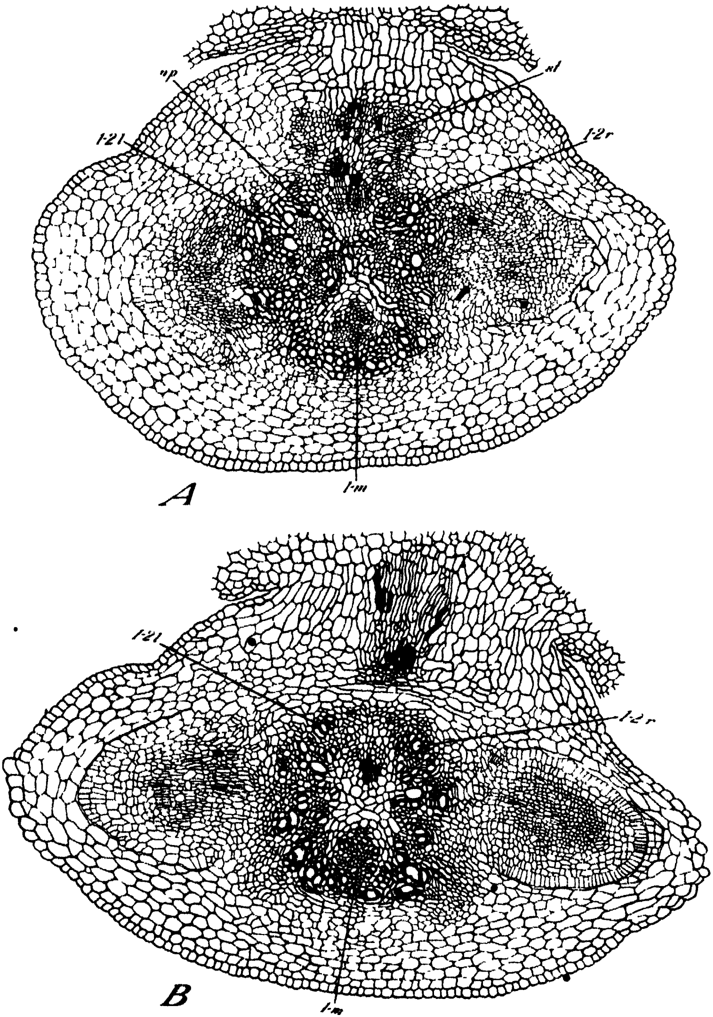


FIGURE 8.—Cross section of the axis (same plant as fig. 5, *B*). *A*, 0.144 mm below figure 7, *B*, in lower part of second node, showing nodal vascular plate, top of arch in scutellum trace, and indications that separation of the second pair of lateral seminal roots from the central stele occurred above this level. *np*, Nodal plate; *st*, scutellum trace; other designations as in figure 5, *B*. × 44. *B*, 0.144 mm below figure 8, *A*, in the first internode, showing central pith, presence of scutellum trace in the stele, complete separation of second pair of lateral seminal roots from stele, and first indication of vascular connections of first pair of lateral seminal roots. Designations as in figure 5, *B*. × 44.

which also makes it easy to trace the vascular primordia in a well-stained section.

In the central procambial system, between the root and stem portions of the embryo, a section of the procambium extends transversely across the axis (fig. 1, *l*). That this procambial tissue separates root and stem is evident. This structure later is interpreted as the first node of the young plant.

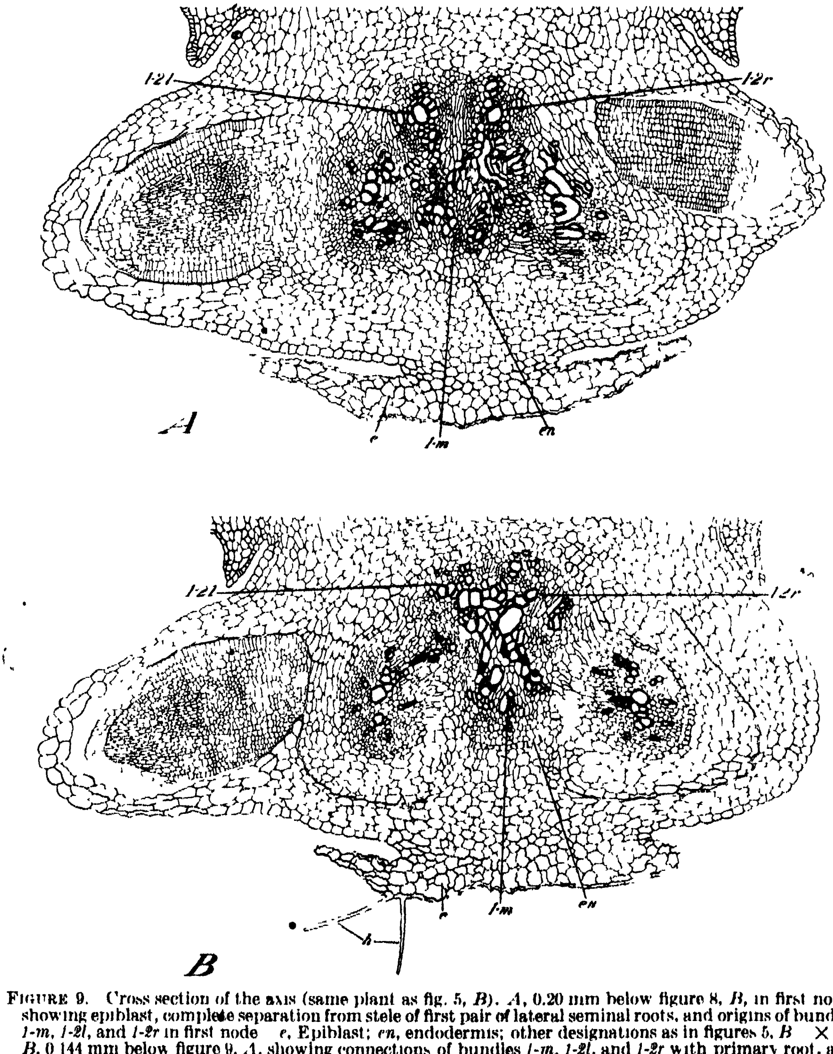


FIGURE 9. Cross section of the axis (same plant as fig. 5, *B*). *A*, 0.20 mm below figure 8, *B*, in first node, showing epiblast, complete separation from stele of first pair of lateral seminal roots, and origins of bundles *1-m*, *1-2l*, and *1-2r* in first node *e*, Epiblast; *en*, endodermis; other designations as in figures 5, *B* × 44. *B*, 0 144 mm below figure 9, *A*, showing connections of bundles *1-m*, *1-2l*, and *1-2r* with primary root, and hair on epiblast. *e*, Epiblast, *en*, endodermis; *h*, hairs; other designations as in figure 5, *B*. × 44.

Extending upward from the cross-axis procambium on the anterior side of the central cylinder is a prominent procambial strand that centers in the well-developed primordium of the first foliage leaf of the plumule (fig. 1, *d*). Similarly, extending upward from the cross-axis structure in the posterior part of the central axis is a procambial strand which, after remaining in the central cylinder for a short space, diverges outward and into the upper portion of the scutellum (fig. 1, *b*).

Slightly above the point at which the scutellum strand diverges from the central cylinder, a small, narrow procambial strand (fig. 1, *j*) extends part way across the axis from front to back. It connects with the back part of the central cylinder but not with the front. A procambial strand extends upward from this cross strand in the back portion of the central cylinder, ultimately passing into the primodium of the second foliage leaf of the plumule. This second cross-axis procambial structure is hereafter interpreted as the second node of the young plant.

The cross-axis structure just above the divergence of the scutellum trace has not heretofore been thought of as a nodal plate. Lermer and Holzner (*31*) show it in their drawing of the barley embryo but say nothing about it. Sargant and Arber (*50*), in their description of the wheat seedling, speak of the root plate in this region but do not suggest that it marks the position of a node.

Between *l* and *j* of figure 1 is a section of the axis bounded by procambial elements in front and back and with what appears to be a central pith parenchyma (fig. 1, *k*). The procambial strand which leads to the scutellum bounds this region on its posterior side, diverging from the axis just under the second cross-axis plate.

A procambial strand leading from the back of the first cross-axis plate downward into the radicle can be identified. In a stained section the procambium is as easily identified in the root as in the stem axis. Identification is not easy without the stain, however, since the shape of the cells of the root procambium is not sharply different from that of the surrounding tissues. There are commonly an odd number (seven or more of each) of alternating xylem and phloem strands in the young root, the xylem being the more prominent. A median sagittal section of an embryo, such as the one represented in figure 1, bisects

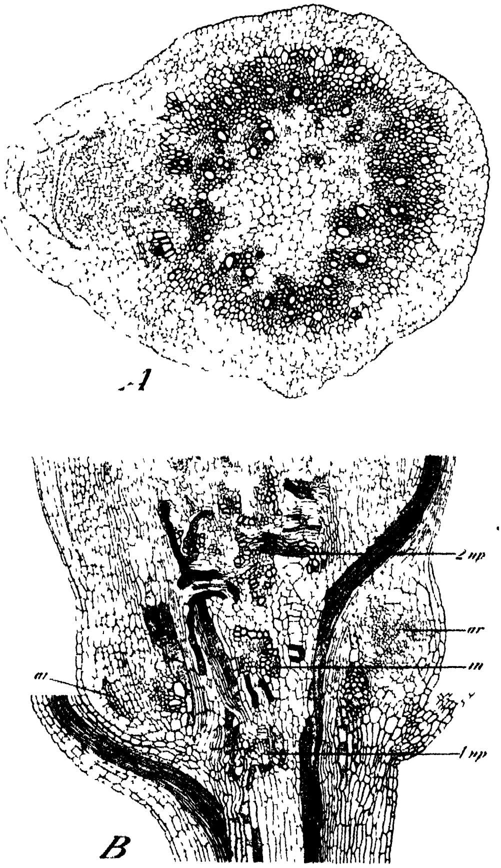


FIGURE 10.—*A*, Cross section of the subcrown internode of a 14-day-old Turkey wheat plant just under the crown node, showing an adventitious root × 44. *B*, A longitudinal section through the first two nodes of the crown in a 14-day-old Hard Federation wheat plant, showing *1-np* and *2-np*, vascular anastomosing nodal plates, leaf-trace divergences, *ar*, root primordia, and *in*, internodes × 32

but one procambial element in the root, which will mature into xylem (posterior side, fig. 1, *l*). The opposite procambial element, which will mature into phloem, is less clearly marked by the form of its cells.

An approximately median longitudinal section through an embryo parallel to its face is shown in figure 2. As is evident from an inspection of figure 1, the orientation of the root and plumule makes it impossible to cut both medianly in the same face section. The section shown in figure 2 is essentially median for the axis proper, although not so for the plumule and not quite so for the primary root. The part of the embryo axis here shown is between the vascular bundles, and the procambium, which will develop into cross-connecting vascular tissue, is not characterized by long, slender cells as is the procambium leading up front and back from *l* in figure 1. It is evidently different, however, from the surrounding pith and cortex parenchyma, and, particularly in a stained section, it can be identified very readily. The two procambial cross-axis structures (fig. 2, *f*, *c*) corresponding respectively to those shown in figure 1, *l*, *j*, are not clear-cut in this view, but they can be identified. The lateral seminal roots, only one of the second pair being present (fig. 2, *d*), plainly originate at or above the procambial plate to which each root belongs.

The lower stem axis region of the embryo changes very little in germination except as its procambium and parenchyma mature into the different tissues of the older plant. Essential features and relationships for this lower region can be fairly well established, even from the limited survey already presented. However, the structure above the second cross-axis plate can be more easily traced after germination and after the development of vascular and other tissues not even suggested in the procambium, etc., of the embryo. This is true especially if there is more than the usual amount of elongation between the second cross-axis plate and the divergence of the coleoptile.

Approximately median sagittal sections through the lower axis region of two 14-day-old plants from just above the divergence of the coleoptile down to and including the transition from root to stem are shown in figure 3, *A* and *B*. The usual amount of elongation in this region is shown in *A*, while much more than the usual amount is shown in *B*. Neither is exactly median, since, even with extreme care it is very difficult so to place a seed that this portion of the axis in the young plant growing from it will be erect and straight. The essential items, however, are shown in both illustrations.

The contrast in elongation between the two plants represented by figure 3, *A* and *B*, shows what may be brought out by growing material under different conditions. In the specimen illustrated in figure 3, *B*, the distance from the under side of the second cross-axis structure (fig. 3, *B*, *k*) to the attachment of the coleoptile at the front of the axis (fig. 3, *B*, *g*) is 1.26 mm. In the specimen on which figure 3, *A*, is based, chosen as representative of the usual response but actually somewhat greater than usual, the corresponding distance (fig. 3, *A*, *k*–*g*) is 0.6 mm. The added length emphasizes vascular structures in this region, although, once recognized, they are perfectly obvious also in the less elongated specimens.

The transition from root to stem (fig. 3, *A*, *n*, and *B*, *n*) is clearly defined. In the embryo it was not easy to place the epiblast definitely with reference to other structures, but in both of these specimens it is clear that the epiblast, *m*, is so attached to the axis as to subtend the cross-axis structure between root and stem proper (fig. 3, *A*, *n*, and *B*, *n*). The divergence of the scutellum bundle from the central axis, as in the embryo, occurs not at the first but at the second cross-axis structure, divergence beginning just under this second plate, *k*. This is clearly shown in figure 3, *B*. The root diverging from the face of the axis (fig. 3, *B*) has its vascular connections at and above this second plate also. The region between the two cross-axis structures (fig. 3, *A*, *n*, *k*, and *B*, *n*, *k*), with its central pith parenchyma, stands out very clearly. The absence of direct connection between the second plate (fig. 3, *A*, *k*, and *B*, *k*) and the front of the vascular cylinder in the median longitudinal plane is more evident than in the embryo.

Above the second cross-axis structure (fig. 3, *B*, *k*), following an intervening space, is a third anastomosing vascular region (fig. 3, *B*, *i*). While that portion of the axis between the second plate and this structure might be interpreted as an elongation of the second plate, such a conclusion seems hardly logical. Cross-axis anastomosing structures are characteristic of nodes, and there are no known cases of elongation in what may be interpreted as nodes in other regions of the plant in any member of the grass family. Considering that, even with the elongation shown in figure 3, *B*, the axis is still much compressed in this region, the separation of these structures is as definite as in the nodes and internodes of the crown (fig. 10, *B*), which latter no one would question. With the evidence of figure 3, *B*, as a guide, the similarities in figure 3, *A*, are not difficult to trace.

It is interesting to note that, in the specimen from which figure 3, *B*, was drawn, the separation of the coleoptile from the axis was 0.92 mm higher on the scutellum side (fig. 3, *B*, *c*) than on the front (fig. 3, *B*, *g*). The presence of the bud in the axil of the coleoptile on the scutellum side of the axis is plain (fig. 3, *B*, *a*). A root primordium on the opposite side of the axis above the coleoptile divergence also can be seen (fig. 3, *B*, *f*).

A description of the vascular anatomy of that part of a 14-day-old wheat seedling from the vascular plate that separates primary root and stem to the separation of the coleoptile from the axis, as shown in serial cross sections, follows.

In the lowest portion of the seedling axis individual bundles are not differentiated, and without some previous hint even the origins of bundles that are easily identified higher up in the axis can be traced only with difficulty. By proceeding downward from the upper sections, vascular elements can be traced and identified more easily.

A transverse section of the plant at a level slightly below that at which the coleoptile is completely free from the axis is shown in figure 5, *B*. At this level there is no connection between axis and scutellum. In the axis there is a central vascular cylinder of 10 definite bundles with an enclosed central pith. The bundles are all collateral with internal xylem and external phloem. The structure is that generally considered typically internodal.

In the central cylinder opposite the scutellum one bundle is larger and more prominent than the others (fig. 5, *B*, *1 m*). When this bundle is traced upward it is identified as the median bundle of the first foliage leaf. On the opposite side and slightly within the circle of the outermost bundle ring is a bundle (fig. 5, *B*, *2–m*) that, traced upward, proves to be the medium bundle of the second foliage leaf. A line bisecting these two bundles divides the axis into two equal parts and fixes the plane of axial polarity, the plane with reference to which the leaves and branches of the main axis are alternately and oppositely placed. The longitudinal procambial origins of these bundles are shown in figure 1, and their longitudinal sections are shown in figure 3, *A* and *B*.

In addition to the median bundles of the first two foliage leaves the vascular cylinder contains six additional bundles in the outer ring (fig. 5, *B*, *1–1l*, *1–2l*, *1–3l*, *1–1r*, *1–2r*, and *1–3r*), three on either side of bundle *1–m*. When these bundles are traced upward they prove to be lateral bundles of the first foliage leaf. Slightly within the outer bundle ring, just as is bundle *2–m*, are two other bundles, *2–1l* and *2–1r*, lying between bundles *1–1r* and *1–2r* and bundles *1–1l* and *1–2l*, respectively. These are the main lateral traces of the second foliage leaf, the other laterals of which are derived by the branching of these and other bundles.

On the same side of the axis as bundle *2–m* and just outside the central bundle ring is a group of bundles which, at this stage of development, are smaller than the central bundles and are somewhat indistinctly differentiated (fig. 5, *B*, *cbt*). These are the vascular connections of the bud which develops in the axil of the coleoptile and is attached to the axis slightly above the level of this section.

In sections from this same region in slightly older seedlings adventitious-root primordia are evident (fig. 5, *A*). These are the roots that have been interpreted as a third pair of lateral seminal roots (*43*) but are not represented by primordia in the embryo (fig. 2). Figure 5, *A* and *B*, shows that these roots develop only some time after germination. However, the fact that these roots develop so definitely in this position is important in identifying this general section of the axis as a node, as will be discussed more fully later.

The two coleoptile bundles (fig. 5, *B*, *cl* and *cr*), one on either side, are situated slightly toward the posterior or scutellar side of the axis. Their upward extension is without particular significance in this consideration other than that they are confined to that part of the coleoptile posterior to the vent (pl. 1, *B*). The possible significance of this position of the bundles will be brought out later.

The coleoptile is almost free from the central axis at the level of this section (fig. 5, *B*). It is still slightly joined, however, at the posterior or scutellar side. In the seedling, sections of which are shown in figures 5, *B*, to 9, *B*, the coleoptile was attached to the axis 0.17 mm higher on the back than on the front (on the basis of the number of intervening sections 12μ thick). Attachment at the sides was lower than at either the front or the back (fig. 6, *A*), that on the left side, facing from the scutellum, being 0.36 mm lower, and that on the right side 0.56 mm lower than at the back. In five embryos the posterior attachment of the coleoptile to the axis averaged 0.18 mm higher than the anterior attachment. In five seedlings the posterior attachment averaged 0.49 mm higher. In the seedling, a section of which is shown in figure 3, *B*, as previously noted, the posterior connection was 0.92 mm higher than the front connection. These differences, though not universal (fig. 3, *A*), seem to be typical.

mary root and in the coleoptile, and, as might be expected, is more rapid in the former. As the food moves into the first node it immediately enters a most efficient cross-axial conducting system, which connects with all bundles of the root axis. The coleoptile, having only two bundles and being dependent on diffusion to a greater degree, naturally grows somewhat more slowly, in spite of its direct connection with the food supply.

It has been pointed out that the first pair of lateral roots are associated with the first nodal plate. In common with the primary root they are in a position to draw directly on the labile food supply conducted by the scutellum trace into the first node. The advantage of this placement is shown by the fact that this pair of roots emerges immediately after the primary root and that they develop in the great majority of cases. The growth of this pair of lateral roots also is almost as rapid as that of the primary root, and all three of these roots ultimately reach much the same degree of development.

The second pair of lateral roots, associated with the second node, is much less favorably situated with reference to food stored in the endosperm. To reach these roots food materials must pass either from the first node, where the requirements of the primary root, the first pair of lateral roots, and, as will be shown later, the first foliage leaf, reduce the total, or through the relatively smaller connections from the coleoptile bundles into the axis. In the latter case the second lateral roots also must compete with such other structures as the coleoptile, the axis itself, the plumule, etc. That the amount of food material available through these channels is not always adequate is shown by the fact that in a large number of cases neither member of the pair develops, while in many others only one grows. When this pair of roots does develop, their slower growth shows the result of their less favorable position.

The direct connection of the median bundle of the first foliage leaf with the first node has been noted. There also is a direct connection across the first node from the scutellum trace to this bundle. It is apparent that any nutritional advantage favoring the rapid development of this first leaf, the first synthesizing organ of the young plant, is advantageous. The vascular structure insures this bundle a favorable connection both with labile food materials from the endosperm and with soil nutrients. The sharp separation of the median bundle from the second node, with such insurance as this may give that no food material shall be diverted from the young leaf at this level, is also undoubtedly significant. In the evolutionary sequence variants possessing this characteristic would seem to have a greater survival potential than others possessing a structure allowing for greater diversion.

The connection of the second leaf and of the growing point with the endosperm food supply through axial connections with the coleoptile bundles is also significant. That these structures are able to divert a considerable part of the food materials conveyed through this channel is indicated by the relatively poor development of the second pair of lateral roots in so many cases.

It is a suggestive fact that the sixth seminal root, located at the second node on the front face of the axis, develops less frequently than the others, and then usually later and less vigorously. Nutritional supplies for this root during the germination period are very evidently less abundant than those for the others. While this root is directly connected with the median bundle of the first foliage leaf (fig. 3, *B*), its later development suggests that the upward nutritional stream from stored sources is not extensively diverted to this root, but rather that its growth is chiefly dependent on material synthesized later by the first leaf.

Taylor and McCall[9] have shown that in seedlings grown at high temperatures, when growth and use of endosperm food supplies both are rapid, the bud in the axil of the coleoptile fails to develop. At low temperatures, when growth is slow, this bud develops in a large percentage of cases. It is very evident that the development of this bud is dependent on the availability of stored food supplies. As noted in figure 5, *B*, the vascular connections of this bud originate from bundles *2–m*, *1–3l*, and *1–3r*. The connections of the coleoptile bundles with the stele (fig. 8, *A*) make rather slight union with the vascular complex from which these bundles originate. When respiration and growth are relatively slow, as at lower temperatures, the stored food is naturally used more slowly, and there is a transfer and secondary storage of food materials in the cortex of the seedling itself. When this condition exists food materials reach bundles *2–m*, *1–3l*, and *1–3r* in such quantity as to induce the development and growth of vascular connections for the coleoptile bud. With more rapid use of stored food the connection is so inadequate that there is an insufficient supply of food materials reaching these bundles. Consequently, the vascular connections for the coleoptile bud are very poorly developed. This is true to such an extent that, even after the crown leaves have grown to a point at which they are synthesizing excess food materials, these materials cannot be utilized by the coleoptile bud because of inadequate primary vascular connections. The presence or absence of adequate vascular connections is apparently the factor that finally determines the development of this bud.

The subcrown root system of the wheat plant does not ordinarily play so large a part in the later life of the plant as does the crown-root system, nevertheless it does play an important role. As previously noted (*33*), under certain arid conditions crown roots fail to develop. The subcrown system in such cases is capable of carrying the plant through its life cycle to complete maturity. The plant does not tiller to any extent under these circumstances, and in many instances only the main culm develops fully. The main culm, in all but size, is entirely normal, however, except for a tendency to lodge because of the absence of the usual anchoring supplied by the crown roots. The connection of the bundles of the primary axis with the primary and lateral roots is direct, and, despite the restricted extent of these roots and the limited cross section of the subcrown axis, the end result demonstrates that roots and axis together are a highly efficient absorbing and conveying system. The observations of Simmonds and Sallans (*54*) and Todaro (*61*) emphasize the importance and efficiency of this system.

DISCUSSION

A fact advanced as a principal reason for interpreting the coleoptile as an extension of the ligule (*7*), the leaf of which the scutellum is the blade, or of the sheath (*2*) of this leaf, is its direct vascular connection to the scutellum trace. Several items of evidence fail to support such a view. Percival (*43*) notes that, in wheat, for example, the ligules and auricles are without vascular elements. The proof offered that even in wheat there is an internode intervening between scutellum and coleoptile divergence suffices to discredit such an interpretation. The supplementary bundles of *Triticum dicoccum*, which do not connect with the scutellum trace, completely clinch the case. If these supplementary bundles were really part of a leaf of which the scutellum is another part, they should originate below the second node and should diverge from the axis at the same general level as the scutellum trace. Actually, these bundles diverge above the second node (fig. 12), thus proving conclusively that the coleoptile is not a part of the scutellum. Percival (*44*) also interprets these bundles as proving the separate identity of the coleoptile, but he bases his opinion on other reasons than the divergence of the bundles above the node of scutellum-trace divergence.

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[9] TAYLOR, J. W., and MCCALL, M. A. See footnote 7.

Differences in the location of intercalary growth in Van Tieghem's (*59*) three embryo types, which have been pointed out, seem to be strong confirmatory evidence in support of the interpretation of embryo structure and homologies herein presented. In the Gramineae, in all portions of the plant above the embryo, intercalary growth occurs only in the internodes. There is no reason to expect this condition to be materially modified in the embryo. The intercalary growth that occurs in *Avena*, below the divergence of the scutellum trace from the axis, in itself indicates that the portion of the axis below this divergence is internode. Similarly, the intercalary growth normally occurring in *Zea* between scutellum and coleoptile divergence argues that this section likewise is internode and not an elongation of the node of scutellum divergence, as maintained by Worsdell (*65*), Howarth (*25*), and others. The latter reasoning also applies to *Triticum*. If these sections of the axis showing intercalary growth are internodes, then scutellum divergence must be from the second node and coleoptile divergence from the third node.

The theory of Sargant and Arber (*50*) that the mesocotyl represents an evolutionary fusion of the stalk of the scutellum with the hypocotyl of a hypothetical epigeous ancestor does not seem to be in accord with the facts. These authors were evidently confused by the structure in *Avena*, the explanation of which has been given above. So far as vascular connections, etc., are concerned, there are no fundamental differences between *Avena*, *Zea*, and *Triticum*. The essential difference between the three genera is in the location of important intercalary growth in one or another of the three lower internodes. There is certainly nothing in the structural development of *Triticum* or of *Zea* to support the fusion hypothesis, nor is there any necessity for so interpreting that of *Avena*.

A theory recently has been advanced by Boyd (*3*) to the effect that the scutellum of the grasses represents the evolutionary fusion of a sucker, such as that of *Hedychium*, with part of a sheathing cotyledon, the latter, in effect, appressed over the face of the former. The anatomical evidence submitted in the foregoing pages does not require such a complicated theory to explain the case. The vascular connections of the scutellum diverge from the axis at a node, exactly as do those of any other leaf. On the basis of leaf relations in the grasses there is no reason to consider the scutellum as anything other than a leaf. It seems unnecessary to develop a theory involving anything more complicated than modification of a leaf to account for the facts.

Boyd (*3*) has called attention to the fact that the term "mesocotyl" is misleading, implying, as it does, location in the "middle" of the cotyledon. In maize the so-called mesocotyl is actually epicotyl, as it is in wheat when it develops. In oats, on the other hand, what is called the mesocotyl is actually hypocotyl. Even though the term be incorrect, some might consider its continuation desirable because of its wide usage. However, no argument can make it seem logical or useful to use an incorrect term synonymously for two entirely different structures. It would seem better to abandon the term entirely and to designate correctly that part of the maize axis heretofore called mesocotyl as epicotyl, and that part in oats called mesocotyl as hypocotyl.

Failure to recognize that the scutellum diverges from the second rather than from the first node has been responsible for much confusion in interpreting the grass embryo. It has been responsible for the opinion that the epiblast is a rudimentary leaf diverging from a second node. It was undoubtedly responsible for the suggestion of so sound an observer as Percival (*43*) that the seminal root on the face of the second node represents a former bud in the axil of the epiblast. Actually, this root is only a third root at the second node, such a root very commonly occurring opposite the median point of the subtending leaf at any node.

In interpreting the grass embryo there has been a tendency to confuse the issue in some degree at least through overemphasis of evolutionary relationships. If all the facts were available, recognized, and properly interpreted, the evolutionary sequence would undoubtedly tell the complete story. Yet, without all the facts, the overstressing of evolution has led some at least to overlook the significance of anatomical features that are present and to think of the grass embryo as much more modified in its structure and parts than is actually the case.

In all questions of interpretation it seems logical to expect that the simplest explanation, in line with known laws, that fits the observed facts is most likely to be correct. Theories that involve structures or development not usual or readily discernible in the family under consideration are very likely to be questionable.

SUMMARY

A study of the crown and upper portions of *Triticum vulgare* shows a definite positional and vascular relationship between nodal vascular plate, leaf and leaf-trace divergence, and root and axillary-bud origins. Leaves diverge from the axis at the nodes. Leaf traces originate from some node in the axis below the node from which divergence occurs, and divergence begins below the nodal plate. Roots originate at the nodes above leaf divergences, and their principal vascular connection is above the nodal plate. Axillary buds originate at the nodes in the leaf axils, also well above the nodal plate. Similar relations prevail in the embryo.

The cross-axis procambial plate separating primary root and stem in the embryo is the first node of the young plant. Associated with

this node are roots and the divergence of the epiblast. Because of its association with a node and with root origins and its alternate distichous position with reference to the next succeeding leaf, the epiblast is interpreted as a vestigial leaf. It is without vascular connection because of its position at the first node, below which is root tissue, in which leaf traces do not originate.

Immediately above the first node is a short internode with enclosing procambial cylinder and central pith parenchyma. Terminating this internode is a cross-axis procambial plate, the second node of the embryo. Immediately under this nodal plate the scutellum trace begins to diverge from the axis. Roots take origin above the nodal plate. Because of its association with nodal plate and root origin, and the divergence of its trace from the axis below the nodal plate, as with any other leaf, the scutellum is interpreted as a leaf, the functional cotyledon, divergent from the second node.

Above the second node is a second internode terminated by a third cross-axis vascular structure. Associated with this structure are the divergence of the coleoptile, and root and axillary-bud origins above the coleoptile divergence. This region is, therefore, interpreted as the third node of the young plant, and the coleoptile as a third leaf. Because of its similarity to the prophylls in function, position with reference to a plumule, outward appearance, and vascular anatomy and connections, the coleoptile also is interpreted as the homologue of the prophyll.

The higher attachment of the coleoptile on that side of the axis toward the scutellum, the vascular relationships of the coleoptile in *Triticum vulgare* and *T. dicoccum*, and Hanstein's observations on the origin of the coleoptile in *Brachypodium* as two opposite projections that later merge into the coleoptile ring, suggest that the coleoptile may represent the evolutionary equivalent of two leaves. In such a case the third node would represent two nodes. If correct, this explains the position of the bud in the axil of the coleoptile on the same side of the axis as the scutellum, when the phyllotaxy of the Gramineae would seem to require it to be on the opposite side. The evidence in support of this hypothesis is considered only suggestive.

The structure of Van Tieghem's three types of grass embryos as exemplified by *Avena*, *Zea*, and *Triticum* are interpreted as being fundamentally similar. In all three the scutellum diverges from the second node and the coleoptile from the third node. The difference between the three lies in the internode location of important intercalary growth during seedling development. In *Avena* this important growth occurs in the first internode, in *Zea* in the second internode, and in *Triticum* in the third internode. The location of intercalary growth in these three embryo types is considered in itself as identifying the internodes and confirming the interpretation of the embryo herein presented.

Embryo and seedling anatomy in the Gramineae does not justify the term "mesocotyl." The structure called mesocotyl in *Avena* is hypocotyl, while that called mesocotyl in *Zea* is epicotyl. The term "mesocotyl" should be abandoned and the correct terms used instead.

Seedling morphology and developmental sequence are determined in large part by vascular anatomy. The primary root, the coleoptile and plumule, and the first foliage leaf are most favorably situated from a vascular standpoint to use food material stored in the endosperm. The first pair of lateral seminal roots also is favorably situated, while the second lateral pair of seminal roots, the face seminal root, and the coleoptile axillary bud are each successively less well situated to use food materials stored in the endosperm.

The primary root system, because of its direct connection with the primary axis, is most effective in absorbing soil nutrients and conducting them to the crown of the plant. Unless destroyed by disease or in some other way, the roots of this system, the seminal roots, remain functional during the life of the plant.

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SELF-FERTILIZATION IN SUGAR BEETS AS INFLUENCED BY TYPE OF ISOLATOR AND OTHER FACTORS[1]

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INTRODUCTION

Self-fertilization in sugar beets (*Beta vulgaris* L.) has been commonly obtained in breeding work by "space isolation" of the plants in gardens and fields. Work of this type has been carried on by investigators in the Division of Sugar Plant Investigations, and lines of sugar beets with records of having been inbred for eight generations are now available. As a means of avoiding off-pollination, it has been the practice since 1929 to have a space of at least one city block separating all plantings.

Certain difficulties are inherent in the space-isolation method of selfing sugar beets. Unless each location is visited frequently for purposes of cultivation, irrigation, and general care of the isolated mother beets, the losses become considerable. Such frequent visits are costly, however, and even with the best of care there is always some loss due to depredation by poultry, dogs, and other trespassers. Unless the irrigation is favorable, the viability of the seed produced may be affected, as shown by Overpeck and Elcock (*9*),[3] who found a direct and positive correlation between irrigations at 1-, 2-, and 3-week intervals and the percentage of germination. Perhaps the most important drawback inherent in space isolation is the danger from cross-fertilization with chard and red beets which have overwintered in gardens with sugar beets in commercial seed fields, or with isolated plantings of sugar beets retained for breeding purposes.

In addition to these difficulties, in using the isolation method one is limited by the lack of suitable locations available. In the work conducted by the writer's colleagues, at Fort Collins, Colo., and vicinity, it was necessary to use 400 to 500 locations as far as 15 miles from the station in order to obtain isolated plantings.

In order to facilitate breeding experiments by the procedure that involves selection in self-fertilized lines, it is exceedingly important to develop some method other than isolation by space, whereby selfed seed in sufficient quantities for breeding work may be produced. The studies reported herein were planned to determine some of the physiological factors affecting seed setting and the comparative value of different types of bags or cages as isolation agents.

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[2] The writer wishes to express his appreciation of the suggestions and criticisms given by A. W. Skuderna, principal agronomist, and G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, during the progress of this work and in connection with the preparation of the manuscript.

[3] Reference is made by number (italic) to Literature Cited, p. 337.

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REVIEW OF LITERATURE

Shaw (*11*) found that the beet flower is self-sterile if its pollen is saved and pollination made later when the stigma is receptive, but that there is no sterility between flowers on the same plant. The flower has been considered protandrous and for that reason the individual flower is ordinarily not selfed. Artschwager (*3*) has found, however, that, in rare cases, the shedding of the pollen and the opening of the stigmatic lobes may occur simultaneously. Wind and insects are considered to be responsible for the transfer of pollen between flowers.

Shaw (*11*) and Artschwager (*3*) consider thrips to be the most active pollenizers.

Reed (*10*) obtained a very small amount of seed from beets which flowered in isolation, but when the plants were covered loosely with a bag which allowed insects to enter, good seed setting was obtained.

Nilsson (*8*) worked out a relatively easy and effective method for selfing sugar beets and mangels. A parchment bag about 5 by 15 inches in size was used. In order to obtain more even ripening, the ends of single branches were cut off. Any lower flowers that were already open were removed and a single branch was then enclosed in each bag. A piece of cotton was wrapped around the stem and the bag tied around the cotton; the upper end of the bag was held out by a little stick fastened to the corners; and both the stick and the lower end of the bag were then tied to a bamboo stake. Good results were obtained when this method was used. The first isolations were made in 1916. Of 168 plants selfed in that year, each of which yielded sufficient seed to obtain a progeny test, 47 to 49 percent produced what appeared to be homozygous progeny.

Vilmorin (*13*) reported the production of sugar-beet seed on mother beets isolated within cloth cages. The cages were shaken each day to assist pollination. Archimovitch (*2*) used cotton-cloth cages with glass windows to cover entire plants in isolation in comparison with vegetable-parchment wrappings on single branches, and obtained better results with the cages. The studies were reported for a 5-year period, and essentially the same results were obtained for each of the last 4 years when comparisons were available. Some evidence was obtained which indicated that the ability of a plant or of individual isolated branches to set seed when isolated under a cage was hereditary in nature.

Stewart and Tingey (*12*) reported results obtained in 1926 from experiments in which they used from 5 to 10 grocery bags of 2-pound size on each of about 75 plants. In some cases single branches were enclosed in a single bag and in other cases several branches were so enclosed. Cotton was not wrapped about the stems in these tests. Striking differences were observed between plants, with respect to their ability to set seed.

Down and Lavis (*5*) reported that the space-isolation method was effective for practical breeding at East Lansing, Mich., and their tests in which vegetable-parchment bags were used on about 30 plants resulted in a total failure to obtain seed. They concluded that this failure may have been due to the very high temperatures that prevailed during the pollinating season.

METHODS AND EQUIPMENT

For the most part, mother beets selected from commercial varieties were used for these studies. A few roots were used that had some inbreeding.

The bags employed for isolation purposes were of several kinds, as follows:

(1) Kraft grocery bags: 12-, 4-, and 2-pound sizes.

(2) Hemp-paper bottomless bags: 12-, 8-, and 4-pound sizes.

(3) Vegetable-parchment bags, large size: Flat bags, approximately 5½ by 17 inches in size, were made by hand from 30-pound parchment paper, waterproof glue being used for the seams.

(4) Vegetable-parchment bags, medium size: 40-pound parchment, gusset style, factory made, 4 by 2½ by 11¼ inches in size.

(5) Vegetable-parchment bags, small size: 40-pound parchment, gusset style, factory made, 3 by 2 by 9 inches in size.

(6) Cellophane bags: Similar in size and construction to the large-size parchment bag, but made of clear cellophane.

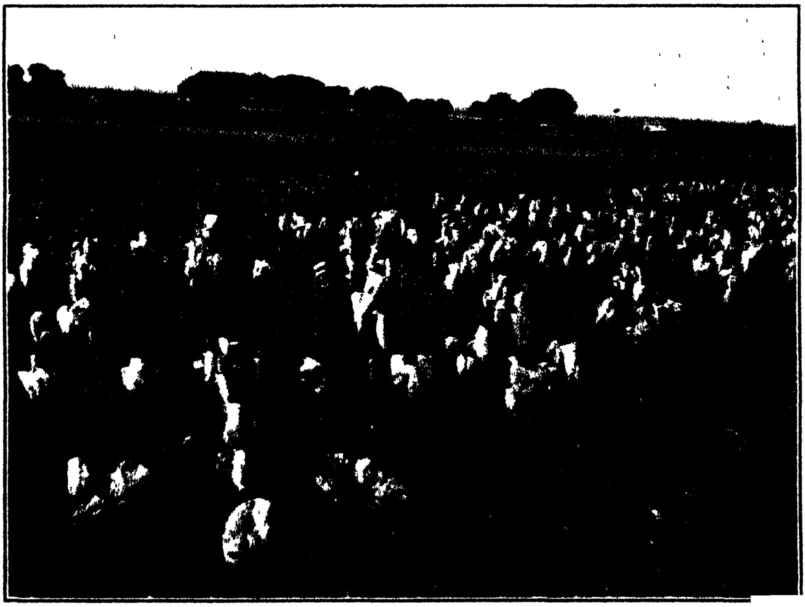

FIGURE 1.—General view of the selfing plot where the different types of bags were used

So far as possible each type and size of bag was used on every beet plant in the test. A general view of the plot used for the bagging studies is shown in figure 1.

In addition, cloth cages of the type employed by Vilmorin were used on some 70 plants. These cages were constructed from a closely woven heavy cotton cloth designated by the manufacturer as 37-inch width, 2.35 yard extra high count drill. The material was preshrunk in a laundry before the cages were constructed. Two widths of material were sewed together lengthwise to form a cylindrical cage, the top being so cut that it could be closed with four seams, as shown by the end view in figure 2. A heavy snap attached to the top of the bag was snapped to a no. 9 wire fastened to the tops of posts, three cages being suspended from the wire between two posts (fig. 3). Two wire hoops, made of no. 8 wire, the ends of which were spot-welded together, were used to keep the cage open (fig. 2). They were held in place by ⅝-inch tape which was sewed at four places, equal distances apart, to the inside of the cage. The lower hoop was placed 18 inches from the bottom of the cage, while the upper one was placed at the point where the top seams began. Two cages were made up with Cello-Glass fronts.

The posts and wire were set-up before the bagging season. Three stakes were driven into the ground at equal distances apart around the plant. A galvanized-iron hoop, 12 inches in diameter, was constructed from a band of material 4 inches in width, with the upper edge turned outward. It was placed over the plant and forced into the ground an inch or more. The cage was very quickly placed in position by attaching the snap to the wire, tying the three pieces of outside tape to the three stakes, and drawing the draw-string up tightly about the metal hoop.

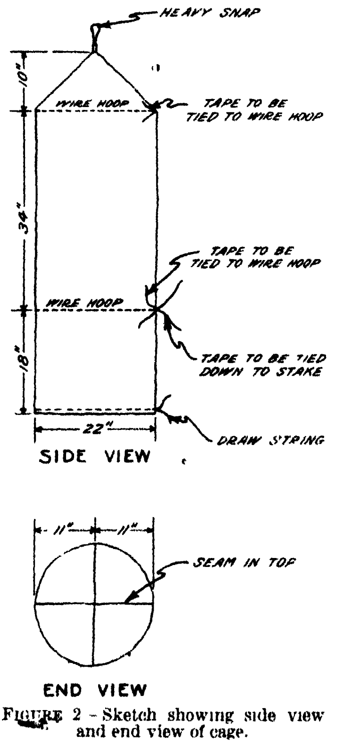
HEAVY SNAP
10"
WIRE HOOP
TAPE TO BE TIED TO WIRE HOOP
34"
TAPE TO BE TIED TO WIRE HOOP
WIRE HOOP
18"
TAPE TO BE TIED DOWN TO STAKE
22"
DRAW STRING
SIDE VIEW
11"
11"
SEAM IN TOP
END VIEW

FIGURE 2.—Sketch showing side view and end view of cage.

In conducting the caging experiment, several red beets were included in the planting and all the beets left to flower were caged. There was little opportunity, under these conditions, for stray pollen to be blown out of one cage and into another, and insects appeared to be effectively excluded. This plan is similar to one used with sweetclover by L. E. Kirk, at the University of Saskatchewan, Saskatoon, Canada.

As a check on the efficiency of the methods of excluding foreign pollen, red beets were also planted at regular intervals in the plot where the bagging studies were conducted. Since any off-pollinations of sugar beets with red beets result in a red F_1 hybrid, it is apparent that this method would afford a test of the effectiveness of the method of isolation.

The branches enclosed in bags were, for the most part, the upper portions of the primary flowering stalks. About the same amount of flowering stalk was enclosed in each bag, except in the 2-pound size; in the latter it was usually necessary to enclose a smaller part of the inflorescence.

The relative humidity of the air enclosed in the various bags and cages was determined by circulating the air through a Selsi precision hair hygrometer in a closed circuit. A hand bicycle pump was used to exhaust the air from the bag and force it over the hygrometer. The screw cap on top of the pump was equipped with airtight packing and an outlet copper tube was brazed into this cap. The hygrometer was enclosed in a galvanized-iron box with a glass front that was soldered and sealed airtight, except the points of connection. This box was connected in the circuit with the pump and bag or cage and the air was circulated through the apparatus (fig. 4). Twenty strokes of the pump were used for the set-up with each bag. The rubber tube connection into the hygrometer and out of the pump on the return to the bag was then closed with clips and the apparatus connected up with the same type of bag on another plant under the

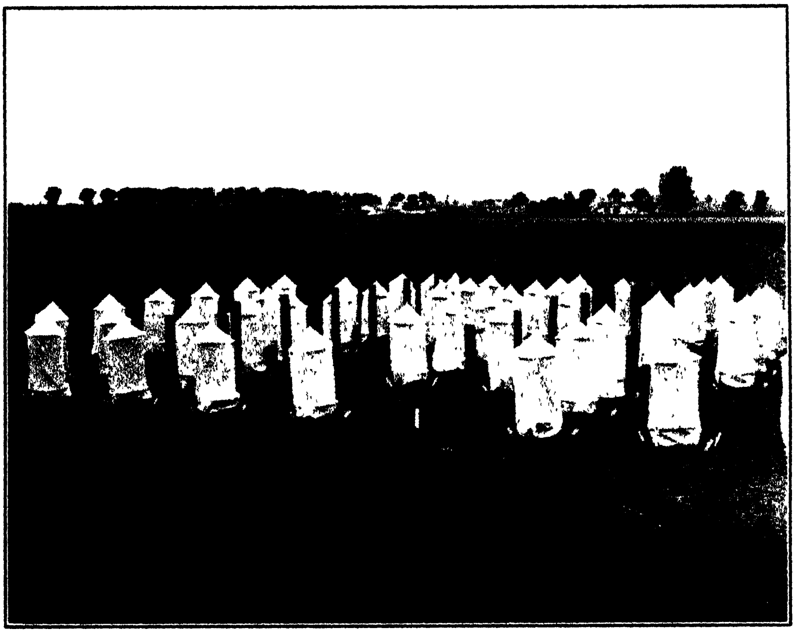

FIGURE 3.—View of caging plot in which individual plants were enclosed in single muslin cages

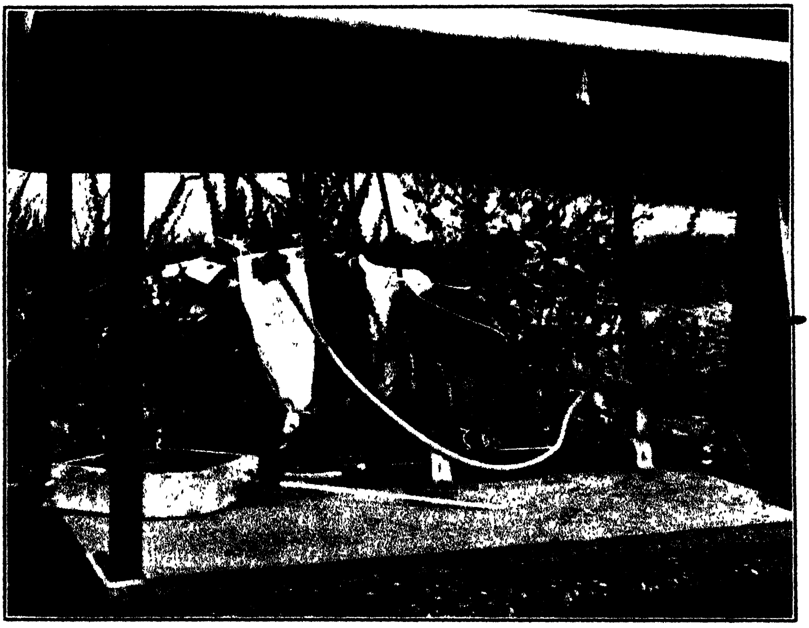

FIGURE 4.—Apparatus for determination of relative humidity. It consists of a hand bicycle pump and hygrometer connected into a closed circuit with a vegetable-parchment bag on a sugar-beet plant.

same type of shade. The total increase on the hygrometer for the two bags was read and recorded.

After the hygrometer was read it was set aside and not used again for 20 minutes or more. After each set a few strokes changed the air in the pump. Six hygrometers were provided for this purpose and used in rotation, but randomized as to the order. Six check readings on the hygrometers were taken before starting the experiment.

TABLE 1.—*Check readings on Selsi hair hygrometers*

Hygrometer no.	Reading no.						Mean	Hygrometer no.	Reading no.						Mean
	1	2	3	4	5	6			1	2	3	4	5	6	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	48	52	64	79	65	51	60	4	44	48	58	72	59	44	54
2	48	59	65	83	66	51	62	5	48	54	62	72	62	48	58
3	48	53	61	73	65	47	58	6	45	52	58	77	60	47	57

It will be observed from these check readings (table 1) that hygrometer no. 2 appears to average slightly higher and no. 4 slightly lower than the others, but the differences are fairly consistent for the six readings. For this particular comparative purpose, these hair hygrometers appear to be very practical. The results obtained seem to be sufficiently accurate for the requirements of this experiment.

In an attempt to obtain readings on the temperature of the air enclosed in the bag a thermometer (fig. 4) was inserted through a rubber cork into the galvanized-iron box containing the hygrometer. Readings taken in this manner did not indicate any material difference between the various bags. A later attempt to obtain accurate readings by inserting the bulb of a thermometer into the bag showed striking differences between bags. Data for the latter method only will be presented. It is probable that as the air was pumped through the apparatus any differences in the temperature of the air among the different bags were neutralized by the hygrometer housing and the pump with its connections.

EXPERIMENTAL RESULTS

RELATIVE HUMIDITY WITHIN BAGS AND CAGES

The relative humidity readings were taken (table 2) in order to compare the different types of bags. For these comparisons, the readings on all bags under a particular type of shade were taken as rapidly as possible, the time consumed for a particular set of bags never exceeding 1 hour. In these comparisons there was an unavoidable error as the air contained in the small bag was diluted to a much greater extent by the air contained in the pump and connections than was the air in a large bag. The branches contained in the small bags, however, were usually similar in size to those enclosed in the large bags.

Table 2 shows that the brown bags, either kraft or hemp, were consistently lower in relative humidity than were the vegetable parchment bags. The muslin cage gave results similar to those of the brown bags. The cellophane bags are comparable in size to the large parchment type, but gave slightly lower average tests for humidity, the difference being of doubtful significance. With but one exception higher humidity values were obtained in medium and small bags constructed of 40-pound parchment than were obtained with the large bags constructed of 30-pound parchment.

TABLE 2.—*Increase in hygrometer readings, resulting from circulating air from 2 bags or from a muslin cage through the hygrometer*

[The comparisons are for different bags on the same plants]

Date (1931)	Hour [a]	Weather conditions			Type of shade	Results for different types of isolators									
						Kraft bags			Hemp bags		Vegetable-parchment bags			Cellophane bags	Muslin cages
		Wind	Clouds	Temperature (° C)		12-pound	4-pound	2-pound	12-pound	4-pound	Large	Medium	Small		
July 2	10 00 a m	Heavy	Slight	30	Lath	4	5	3			9	14	17	18	11
	8 15 a m	do	Clear	28	None	7	7	8			8	41	12	14	4
	11 10 a m	do	Slight	30	Tobacco cloth	2	5	13			19	21	16	24	16
	1 10 p m	do	do	31	Cheesecloth	6	4	7			8	20	15	17	7
	2 25 p m	do	Clear	31	Lath	4	4	8			23	17	20	17	4
	2·30 p m	do	do	31	None	9	13	6			25	37	39	18	3
July 3	10.30 a m	Slight	Slight	32	Lath	3	1	6			12	15	10	11	14
	6·10 p m		Clear	23	None	18	13	5			9	18	12	20	6
	11 00 a m	Medium	Slight	33	Tobacco cloth	3	7	7			14	12	20	19	15
	9 45 a m	do		29	Cheesecloth	1	1	5			8	7	15	7	16
	3.55 p m	do	Heavy	32	Lath	5	2	1			2	7	18	8	15
	3 15 p m	do	do	33	None	3	4	7			6	10	10	10	6
July 5	10 15 a m	Slight to medium	Clear	28	Lath	2	3	6	2	2	14	24	15	16	
	10 50 a.m	do	do	30	Tobacco cloth	2	5	8	2	3	13	12	22	33	
July 6	8 30 a m	Slight	do		None	3	4	6	2	4	24	25	25	18	13
	9 35 a m	do	do		do	4	8	10	6	2	20	21	30	12	9
	10 40 a m	do	do		do	7	10	11	4	4	20	24	26	12	6
	1 15 p m	do	do		do	12	8	11	6	3	24	25	19	17	5
	2 25 p m	do	do		do	9	10	9	7	3	40	20	32	15	7
	3 30 p m	do	do		do	16	12	7	4	6	29	17	19	27	10
	4 40 p.m	do	do		do	10	6	7	5	5	16	22	18	16	7
July 7	7·15 a m	do	do		do	9	8	6	4	3	27	25	24	19	4
Mean of all						6.3	6 4	7 1			16 8	19 7	20 2	16 7	
Mean of July 5 and 6						7 4	7 4	8 1	4.2	3 5	22 7	21.5	24 0	18 5	
Mean of all but July 5						6 8	6.6	7 2			17 2	19.9	20 4	16.0	8.0

[a] When the comparisons were started. From 25 minutes to 1 hour was required to complete the readings on 1 series.

TABLE 3.—*Increase in hygrometer readings resulting from circulating air from 2 bags or from a muslin cage through the hygrometer*

[The comparisons are for different types of shading]

Date (1931)	Hour	Weather conditions	Type of shade	Results for different types of isolator									
				Kraft bags			Hemp bags		Vegetable parchment bags			Cellophane bags	Muslin cage
				12 pound	4 pound	2 pound	12 pound	4 pound	Large	Medium	Small		
July 4	9.40 to 11.45 a. m.	Few clouds, 25° C., windy	None	10				2	24	30	17	18	2
	Do	do	Lath	2	5	4		[illegible]	15	24	19	13	9
	Do	do	Tobacco cloth	4	4	[illegible]	3	0	15	15	10	24	2
5	11.25 to 12.35 a. m.	Clear, 32° C., slight breeze	None	12	12	4		4	24	27	32	15	5
	Do	do	Lath	3	3		[illegible]	1	10	30	16	15	7
6	8.50 to 9.55 a. m.	Clear, 25° C., slight breeze	None	16	[illegible]				18	43	26	17	12
	Do	do	Cheesecloth		4	1			13	11	17	22	9
7	8.20 to 9.35 a. m.	Clear, 20° C., slight breeze	None	11	15	[illegible]	4	3	17	24	28	23	4
	Do	do	Tobacco cloth		3			[illegible]	10	10	14	13	10

For the shading comparisons (fig. 5) the readings on a particular bag and shade were taken and followed immediately by readings on the same type of bag without shade. The data obtained for this comparative study are summarized in table 3.

It was possible to make paired comparisons by Student's method (*1*) to determine the significance of the differences between the humidity values obtained for no shade (check) and for lath, cheesecloth, or tobacco-cloth shading. Table 4 shows results obtained for these comparisons, based on Fisher's *T* test (*6*) for significance. While shading appears to result in somewhat lower average humidity values for each of these comparisons, only the mean of the lath versus check readings for July 4 and 5 gives a *P* value less than 0.05, the difference required for the 5-percent level of significance.

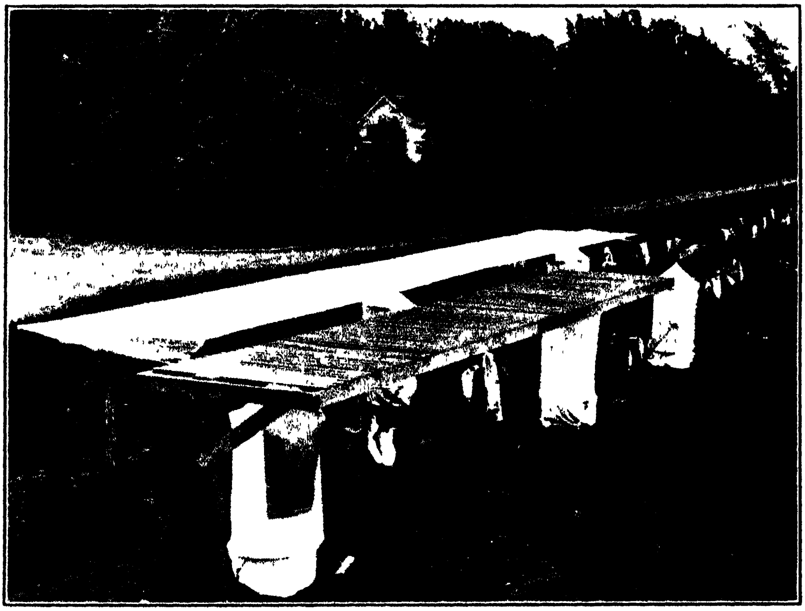

FIGURE 5.—View of plot used for relative-humidity and temperature determinations. In the foreground is the lath shading; in the background, the cheesecloth and tobacco-cloth shading.

TABLE 4.—*Paired comparisons of relative humidity values according to Student's method*

Date (1931)	Shade comparisons [a]	
July 4	Check with lath	Between 0.2 and 0.1
July 5		Between .1 and .05
Mean		Between .05 and .02
July 4	Check with tobacco cloth	Between .3 and .2
July 7		Between .2 and .1
Mean		Between .1 and .05
July 6	Check with cheesecloth	Between .1 and .05

[a] Paired comparisons were made by Student's method (*1*) to determine the significance of the differences between the humidity values obtained for no shade and for lath, cheesecloth, or tobacco-cloth shading.

TEMPERATURE WITHIN BAGS AND CAGES

A small hole was made in each bag and the bulb of a thermometer inserted inside, the thermometer being attached to a bamboo stake. A different thermometer was used for each bag. The bags and cages were unshaded. Readings were made once each hour. A shaded, uncovered thermometer was used as a check. The summarized data are presented in table 5.

TABLE 5.—*Temperature readings with the thermometer bulb inside the bag or cage, as compared with check readings on a shaded uncovered thermometer, July 7, 1931* [a]

Hour [b]	Check	Temperature (°C) within type of isolator indicated									
		Kraft bags			Hemp bags		Vegetable-parchment bags			Cellophane bags	Muslin cage
		12-pound	4-pound	2-pound	12-pound	4-pound	Large	Medium	Small		
8 a.m.	19.6	27.3	32.9	25.6	33.1	29.4	27.1	25.5	25.4	23.1	23.9
9 a.m.	20.6	28.8	30.9	27.6	34.1	30.9	29.6	27.0	26.4	27.1	25.9
10 a.m.	20.6	31.8	30.4	29.6	36.1	30.4	31.6	27.5	26.9	28.1	27.9
11 a.m.	21.6	33.8	28.9	30.6	35.6	28.9	32.1	28.0	26.9	28.1	29.4
12 a.m.	23.6	35.3	28.9	30.1	34.6	26.9	30.1	27.5	25.9	27.6	29.9
1 p.m.	26.1	35.3	28.9	28.6	33.1	28.4	29.1	28.5	26.9	30.1	30.9
2 p.m.	27.1	34.8	29.9	30.6	30.1	30.4	28.1	29.0	28.4	31.6	32.4
3 p.m.	28.1	32.8	30.4	31.6	30.1	31.4	27.6	30.5	28.4	32.6	32.4
4 p.m.	28.1	28.8	31.4	31.6	30.1	32.4	27.1	30.5	26.4	32.6	31.4
5 p.m.	28.1	28.3	30.9	30.6	30.6	31.9	28.1	31.0	25.9	32.6	28.9
Mean temperature	24.4	31.7	30.4	29.7	32.8	30.1	29.1	28.5	26.8	29.4	29.3

[a] All bags were on the same plant, and the muslin cage was on an adjacent plant. The bagged plant had flowered to the extent of about 75 percent. The values given in the table have been corrected in relation to a standard, so that they represent actual comparable temperatures for the various treatments.
[b] Brilliantly clear all day; slight to medium breeze.

The differences in temperature within the various bags were not large. However, the temperatures in all the bags showed some increase over the temperature of the exposed check, and the temperatures within the kraft and hemp bags were higher than those within the vegetable-parchment and cellophane bags or the muslin cage.

SEED SETTING

The summarized data for the bagging and caging studies are presented in tables 6 and 7. Seed counts were made after the seed balls had been cleaned by use of a draper and hand sieves. The seed balls appeared to have good germs.

TABLE 6.—*Percentage of plants setting seed under different types of bags*

Type of plant	Percentage of plants setting seed in type of bag indicated									
	Hemp			Kraft			Vegetable parchment			Cellophane
	12-pound	8-pound	4-pound	12-pound	4-pound	2-pound	Large	Medium	Small	
Commercial variety	42	48	30	50	38	30	87	8	7	17
Some inbreeding	0	50	....	74	44	38	95	15	6	0

TABLE 7.—*Seed setting obtained for all bagged and caged plants*

Seed produced per plant	Bagged plants				Caged plants			
	Commercial variety		Some inbreeding		Commercial variety		Some inbreeding	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
None	96	47.5	8	25.8	5	11.4	1	25.0
Less than 30 seeds	53	26.2	8	25.8	12	27.3	1	25.0
From 30 to 99 seeds	25	12.4	4	12.9	6	13.6	0	------
100 or more seeds	28	13.9	11	35.5	21	47.7	2	50.0
Total	202	100.0	31	100.0	44	100.0	4	100.0

On commercial varieties, from 30 to 99 seed balls per plant were obtained on 12.4 percent of the bagged plants and on 13.6 percent of the caged plants. One hundred or more seed balls per plant were obtained on 13.9 percent of the bagged plants and on 47.7 percent of the caged plants. Some seed balls were obtained on 52.5 percent of all bagged plants and on 88.6 percent of all caged plants. A much higher percentage (74.2) of seed setting was obtained on the bagged plants that had some inbreeding. This difference indicates that selection in inbred lines tended to isolate material which was genetically more capable of producing seed through forced self-fertilization. It is further supported by the fact that certain plants in both groups appeared to set seed under different types of bags and cages much more freely than others (fig. 6). A similar conclusion was reached by Nilsson (*8*).

In order to prevent the entrance of thrips or other small insects cotton was used about most of the stems of branches enclosed in bags. The mouth of the bag was then tied firmly about the cotton. All the bags on 19 plants were purposely left without cotton. Table 8 shows a comparison between seed setting following the use of cotton about the stems and seed setting when cotton was not used. All bags on the plants regardless of type were considered in this study.

TABLE 8.—*Seed setting with and without the use of cotton*

Treatment	Total plants	Plants setting some seed		Plants not setting seed	
	Number	*Number*	*Percent*	*Number*	*Percent*
With cotton	172	80	47	92	53
Without cotton	19	7	37	12	63

Of the plants bagged without cotton about the stems, 37 percent set some seed as contrasted with 47 percent for those bagged with cotton. Had insects entered where the cotton was lacking, quite the opposite result would have been expected. The fact that a markedly higher percentage of seed setting did not result from failure to use cotton seems to indicate that any insects that might have entered the bag where cotton was not used did not constitute an effective means of pollen distribution between plants under the conditions of this test.

A germination test and a seedling study were made on the seed balls from plants which set upwards of 100 or more seed balls. These results are summarized by classes in table 9.

The results obtained show fairly well the efficiency of the methods used to exclude foreign pollen and limit the pollination among the flowers inbred. From a total of 1,350 seed balls, representing 50 each for 27 bagged plants, 721 seedlings were obtained. Not a single plant appeared to be a hybrid with a red beet. Cotton was used about the stalks for all the 27 bagged plants represented. Out of 269 seedlings obtained from 690 seed balls from 23 caged plants, 2 seedlings appeared to show all the characteristics of F_1 crosses with red beets. While these may have been due to foreign pollen carried out of one cage and into another by wind or insects, it is believed more likely that they resulted from cross pollination before the cages were

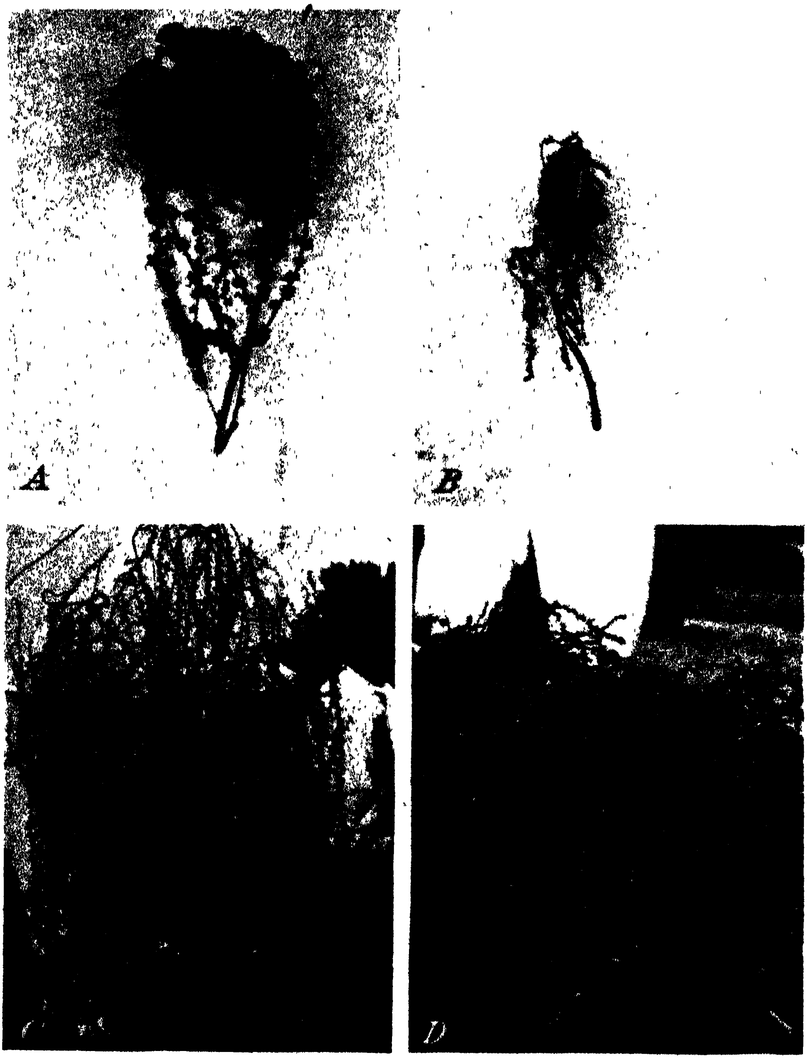

FIGURE 6.—*A*, Branch that when covered with a 4-pound hemp-paper bag produced more than 400 good seed balls; *B*, branch that when covered with a cellophane bag produced no seed; *C*, close-up showing seed on plant that produced an excellent crop of seed under a cage; *D*, close-up of plant showing wealth of inflorescence but practically no seed produced under a cage.

placed in position. The first florets to open are those situated low on the main stalk or in the axils of the branches and are easily overlooked, even after very careful examination. The cages were placed in position on each plant only a day or two before the opening of the earliest flowers on the plant.

TABLE 9.—*Summary of greenhouse germination test on selfed seed*

[Fifty seed balls each were used from bagged plants and 30 each from caged plants]

Type of isolator	Number of plants falling in indicated class based on number of seedlings per 100 seed balls										Mean germination
	0	1-20	21-40	41-60	61-80	81-100	101-120	121-140	141-160	Total	
											Percent
Bags	1	7	5	1	6	4	1	1	1	27	39.0
Cages	3	8	1	4	4	3				23	39.0

The mean germination percentage was 39.0 for seed from both bags and cages. About 50 percent of the plants fell in classes ranging from 41 to 160 seedlings per 100 seed balls. By planting rather heavily, it would be possible to carry as breeding material nearly all the progeny lines represented in table 9. From the practical standpoint, however, assuming poor germination to be possibly heritable, such lines would be discarded.

Of the several types of bags used in the study (table 6), the large vegetable-parchment bags appeared to give the best results, and the medium and small parchment bags proved to be the most unsatisfactory. The kraft and hemp bags were about equally desirable. Of 30 cellophane bags used on 29 plants, seed was obtained on 5 plants, only 2 of which gave more than 5 seed. These 2 yielded 105 and 120 seed, respectively. It is very likely that these seed may have been the result of off-pollination, since the glue used did not hold satisfactorily on the cellophane bags. At harvest, these bags were carefully checked and those showing a loose seam were discarded. In the few exceptional cases a faulty seam may have escaped notice.

Under the conditions of this experiment, the differences in temperature and relative humidity of the air contained in the bags and cages appeared to be of doubtful value in explaining the differences in seed setting. The use of brown bags, both kraft and hemp, resulted in low humidity and high temperature within the bag, but rather good seed setting. The use of parchment and cellophane bags resulted in comparatively high humidity and low temperature, and low seed setting, with the exception of the large parchment bags, the use of which resulted in the highest seed setting of all the bags tested.

Cello-Glass, which was very easily sewed directly to the heavy drill cloth, was used in the construction of two cages (fig. 5). The plants appeared to grow quite normally inside these cages, just as they did in many of the regular cages. It is doubtful whether the use of Cello-Glass windows in the cages was of any value under the conditions of this experiment. Owing to the extreme genetic variability among the mother beets used for these studies, the seed setting on these two plants is of no value in estimating the efficiency of Cello-Glass windows for cages.

One plant was cut back to a single main stalk and a cage was used to cover only the stalk. The foliage about the crown was not covered and appeared quite normal throughout the season. The seed setting on this plant was very poor.

DISCUSSION

In the absence of pertinent genetic data, it may be assumed, for theoretical purposes, that the ability to produce seed under conditions of forced self-fertilization is hereditary in nature. The data obtained in this study tend to support this theory. In ability to produce viable seed under bags or a cage, there were striking differences among plants obtained from commercial varieties; and the plants with some inbreeding were capable of producing more selfed seed than the selections from commercial varieties.

With regard to seed setting when plants are bagged, the results obtained in this study on sugar beets are very similar to those obtained in studies on rye by Heribert-Nilsson (*7*) and Brewbaker (*4*). The conclusion was reached by the latter that the characters of high and low self-fertility appear to be heritable and that by selection and continued self-pollination highly self-fertile lines can be obtained.

Assuming also that there are genetic factors conditioning the viability of the seed produced, it is to be confidently expected that by continued selfing and selection in self-fertilized lines the strains isolated should become relatively more easy to continue, as the poorer seed producers and factors for low viability are discarded. This will be all the more true if, as Nilsson (*8*) found for certain lines, appreciable loss in vigor results from inbreeding.

SUMMARY AND CONCLUSIONS

A study was made of the relative effect of various factors on seed setting in sugar beets under conditions of forced self-fertilization.

Ten different types of bags were used. So far as possible, each type of bag was placed on every plant. A large hand-made bag, about 5½ by 17 inches, constructed of 30-pound vegetable parchment, gave the most promising results, 87 percent of the bags containing some seed. The 12-pound and 8-pound hemp and the 12-pound kraft bags contained some seed on 42, 48, and 50 percent, respectively, of the plants so bagged. The 4-pound hemp and 4-pound and 2-pound kraft bags were next in order of promise, while plants enclosed in factory-made medium bags and in small-size parchment and cellophane bags yielded very poor seed setting. For plants with some inbreeding the seed setting was somewhat higher in each case, but the comparative values were similar.

A specially constructed muslin cage was used in an isolated plot, all plants in the plot being caged. Of about 44 plants caged, only 11.4 percent failed to set seed, the others ranging from poor to practically perfect seed setting. This difference was, in all probability, hereditary in nature.

Wide differences were obtained in the relative humidity of the air exhausted from the different bags. The relative humidity for the large parchment bag appears to have been about the same as that for the medium and small parchment bags, but was much higher than for the brown hemp or kraft bags. The differences in this respect do not correlate with differences obtained in seed setting.

When the sun was shining, the temperature within the brown bags was higher than it was within the white bags. There was no correlation in this respect with the differences in seed setting

Two specially constructed cages having Cello-Glass fronts were used in the experiments. In another instance, a plant was entirely enclosed in a cage, with the exception of the crown leaves. No particular advantage appeared to result from the use of either of these special methods.

The results obtained indicate that some method of isolation in which either bags or cages are used may prove entirely practicable in intensive breeding and genetic work with sugar beets.

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MAIZE CROSSING VALUES IN SECOND-GENERATION LINES[1]

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INTRODUCTION

Puerto Rican farmers are unfamiliar with the use of maize lines inbred through many generations for the production of first-generation hybrid seed. The aim of the breeding program of the Federal Agricultural Experiment Station at Mayaguez has been to test crossing values of lines inbred through the relatively short period of 2 or 3 generations; it was thought that their reasonably good productivity would facilitate their use under local conditions. Two methods have been devised for such tests, line-variety and line-recessive yield trials. The hybrids used in the line-recessive yield trials were produced by pollinating the more vigorous lines with a line assumed to be recessive because of its white kernel color and inferiority in plant size, ear size, and yield. The object of the work described in this paper was to determine whether increased yields could be secured by outcrossing lines inbred only two generations and whether the crossing values of these lines could be tested by either of the foregoing methods.

REVIEW OF LITERATURE

No data have been noted in the literature covering the use of the line-recessive method. Jones [3] reported on line-variety hybrids in 1921 though not as a method of testing lines. Yield trials with line-variety crosses and their suggested use as a basis for elimination in selfed lines were briefly reported on in January 1929 by the Mayaguez Station.[4] Since that is thought to be the first published suggestion of the use of this method and in view of the marked interest shown at present on line varieties this report is quoted in full as follows:

> Crosses were made between a common open-pollinated variety of native corn and 27 lines of corn originating from high-yielding Porto Rican parent ears. These lines had only been self-pollinated twice, but it was thought that the best lines for additional selfing could be determined by comparing the yields of the hybrids with the yields of a common pollinator. Most of the hybrids were expected to equal and a few slightly to exceed in yield the ordinary native corn, which was used as the pollen parent. The results were very encouraging. The average yield of the hybrids was approximately 20 percent greater than that of the check variety or pollen parent. Taken as a whole, the ears of the hybrids had very sound grain, and were much freer from mould and from worm injury than the ears of the check. The hybrid plants were sturdy and lodged less frequently than did those of the check variety.

Lindstrom [5] reported significant increases in yield over open-pollinated parent varieties through the use of line-variety crosses.

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[3] JONES, D F. THE PRODUCTIVENESS OF SINGLE AND DOUBLE FIRST GENERATION CORN HYBRIDS. Jour. Amer. Soc. Agron 14: 246. 1922.

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Jenkins and Brunson [6] found significant correlations between yields of inbred lines crossed inter se and the same lines in line-variety combination; they concluded that crosses with open-pollinated varieties may be used efficiently in preliminary testing of new lines.

The data presented in this paper differ from those already published in full by Lindstrom, and Jenkins and Brunson, in that they are based on less homozygous material, only two generations of inbreeding being practiced prior to crossing. Most of the parent-progeny correlation studies reported on in the literature are on lines inbred from 5 to 6 generations, but Jenkins [7] reported on less homozygous material and found high-yielding values in inbred lines in their F_1 crosses indicating that even after only 3 or 4 generations of selfing lines must be homozygous for many of the factors that make for yield. He reports also significant and positive correlations between yield of the F_1 crosses and most of the characters in the inbred line that make for plant vigor; these correlations indicated that the most productive crosses may be expected from the most productive inbred parents.

METHODS OF EXPERIMENTATION

The maize used for breeding material was collected from many districts in Puerto Rico, and the less desirable ears were eliminated. The foliage weight of 20 to 25 seedlings at 3 weeks was compared with the weights of other cultures grown in the same germination flat, and parent ears were retained which produced large dark-green seedlings. The highest yielding parent ear from each district as determined in hill-checked ear-row trials was inbred. The process of selection in selfed lines was essentially the same. Lines were favored which compared well with open-pollinated corn in plant growth.

Since a similar reaction or parallel performance among related lines in hybrid combination would afford strong evidence that early elimination is justifiable, the inbred material tested comprised a number of groups of sibs. In order to include a wide range of breeding stock, lines were chosen from unrelated parent ears each one of which originated in a different district. Thus, although only 51 lines are reported on, containing 10 groups of second-generation sibs, they were derived from 8 parent ears entirely unrelated to each other. Taken as a whole, they are fairly representative of the corn for the entire island.

When elimination is practiced early in the process of selfing and the aim is to retain only high yielding lines with large plant growth, it is particularly important to guard against contamination during hand pollination, as hybrid plants are easily confused with selfs. The bag withdrawal method was devised for this purpose. The well-known bottle method was used, but instead of removing the ear bag upward, as is customary, thus tending to expose the silks, it was ripped open and pulled straight downward after the tassel bag and the tassel were in position. The use of the bag-withdrawal method enables the worker to remain 4 to 6 inches from the silks and safeguards them against being touched by the hand and against contamination with foreign pollen.

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[6] JENKINS, M. T., and BRUNSON, A. M. METHODS OF TESTING INBRED LINES OF MAIZE IN CROSSBRED COMBINATIONS Jour. Amer. Soc. Agron. 24: 523-530. 1932.

[7] JENKINS, M. T. CORRELATION STUDIES WITH INBRED LINES AND CROSSBRED STRAINS OF MAIZE. Jour. Agr. Research 39: 677-721. 1929.

In producing the hybrid seed it has been the practice when detasseling to destroy the short plants which are easily overlooked and might otherwise shed contaminating pollen. Late-flowering plants of the pistillate parents were topped just above the upper ear in order to reduce supervision and again to lessen the chance of contamination; the cost of hybrid-seed production is also reduced by this operation as some plants may be 6 to 8 days late in tasseling and when left nearly double the detasseling period.

In all the yield trials reported herein a control or check variety was planted in every other or every third plot. The plots were long and narrow, either 1 or 2 rows wide. It is thought that the direct comparisons on yields with those of adjoining checks plots were fairly reliable and that the correction for variations in drainage and soil moisture thus secured more than offset the effect of intervarietal competition.

For determining the significance of yield differences Love's modification of Students' method was used.[8]

EXPERIMENTAL DATA

YAUCO-TORRE LINE-VARIETY YIELD TRIALS, MAYAGUEZ, 1927

Yauco-Torre corn, a completely heterozygous open-pollinated variety, and 27 line-variety hybrids produced at Mayaguez in 1926 were used in an experiment to determine the yields of second-generation inbred lines when top crossed by Yauco-Torre. The staminate parent was an open-pollinated variety from Yauco. The pistillate parents were 27 second-generation lines derived from 3 high-yielding parent ears from Lajas, Peñuelas, and Jayuya. The seed was stored in sealed containers with quicklime during the winter season. Each hybrid consisted of seed from 35 to 50 ears bulked together.

Planting was heavy, 4 to 5 seeds per hill. The stand was thinned to 1 plant per hill and consisted of 10,900 plants per acre. Each 1-row plot consisted of 24 plants and was one five-hundredth of an acre in area. Every other row was planted to the check variety. A very heavy wind and rain storm toward the end of the crop season caused considerable lodging in the check corn but did little damage to the hybrids; as a result there was somewhat more decay in the check than in the hybrid ears.

Four plots were grown. In plot E each variety was planted in a single row and compared directly with an adjoining row of the Yauco-Torre corn. In plot F each variety was repeated, but no checks were grown on account of the limited area of land available. In plots K and L each line variety was grown once in comparison with adjoining check rows of Castillear-1-5-1 × Yauco-Torre, one of the more promising hybrids. In plot E there were 26 replications of the Yauco-Torre check corn. In plots K and L the line-variety check was replicated 52 times.

Yields per acre of the various plots are given in table 1 in bushels of air-dry shelled corn. The moisture content of the various hybrids was assumed to be the same as none of the corns was contrasting in kernel type. This procedure probably gave a slight advantage to the Yauco-Torre corn which was in general a somewhat softer type of corn and consequently higher in moisture content.

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[8] LOVE, H. H. A MODIFICATION OF "STUDENTS" METHOD FOR USE IN INTERPRETING RESULTS. Jour. Amer. Soc. Agron. 16: 68-73. 1924.

TABLE 1.—*Yields of air-dry shelled corn (bushels per acre) in the Yauco-Torre line variety-hybrid trials, 1927*

Hybrid	Plot E			Plot F	Plot K			Plot L			Average increase over check in plots K and L	Average of 4 plots of hybrids
	Hybrid	Average of 3 or 4 nearest Yauco-Torre check rows	Increase(+) or decrease (−) as compared with check	Hybrid	Hybrid	Average of 3 nearest Castillear-1-5-1×Yauco-Torre check rows	Increase(+) or decrease (−) as compared with check	Hybrid	Average of 3 nearest check rows of Castillear-1-5-2×Yauco-Torre	Increase(+) or decrease (−) as compared with check		
Castillear-1-5-1×Yauco-Torre	63.5	47.5	+16.00	54.95								
Castillear-1-5-2×Yauco-Torre	43.8	36.9	+6.90	46.50	54.9	48.83	+6.07	33.8	47.55	−13.75	−3.84	44.80
Castillear-1-3-1×Yauco-Torre	38.1	33.23	+4.87	43.90	58.6	48.12	+10.48	45.3	43.65	+1.65	+6.07	46.48
Castillear-1-1-1×Yauco-Torre	56.5	32.80	+23.70	48.30	45.5	47.20	−1.70	40.0	47.90	−7.90	−4.80	47.60
Vicens Flint-2-2-2×Yauco-Torre	42.2	32.10	+10.10	35.70	40.0	47.51	−7.51	32.7	44.55	−11.85	−9.68	37.65
Vicens Flint-2-9-1×Yauco-Torre	37.5	26.33	+11.17	39.15	36.4	48.53	−12.13	34.8	45.10	−10.30	−11.21	36.96
Vicens-21-1-3×Yauco-Torre	40.1	26.7	+13.40	23.50	40.0	45.23	−5.23	40.3	43.30	−3.00	−4.11	35.97
Vicens Flint-2-14-2×Yauco-Torre	31.7	26.4	+5.30	31.20	38.6	45.50	−6.90	33.8	44.70	−10.90	−8.90	33.83
Vicens Flint-2-12-1×Yauco-Torre	49.6	27.1	+22.50	49.2	41.7	43.52	−1.82	35.9	42.15	−6.25	−4.03	44.10
Vicens Flint-2-4-1×Yauco-Torre	36.0	28.2	+7.80	57.0	42.2	44.75	−2.55	35.4	43.70	−8.30	−5.42	42.65
Cacique-1-2-1×Yauco-Torre	41.2	26.8	+14.40	53.15	44.9	42.80	−2.10	35.4	45.65	−10.25	−4.07	43.66
Cacique-1-2-4×Yauco-Torre	46.5	24.4	+22.10	48.65	54.9	45.40	+9.50	45.9	47.15	−1.25	+4.13	48.99
Cacique-1-50-1×Yauco-Torre	32.8	25.6	+7.20	35.65	30.6	45.5	−14.90	33.2	44.7	−11.50	−13.20	33.06
Cacique-1-67-1×Yauco-Torre	35.4	25.6	+9.80	47.80	41.7	49.55	−7.85	41.7	43.2	−1.50	−4.67	41.65
Castillear-1-4-1×Yauco-Torre	30.6	21.3	+6.30	45.45	32.8	46.50	−13.70	28.5	44.8	−16.30	−15.00	34.34
Castillear-1-4-3×Yauco-Torre	44.0	26.74	+17.26	59.60	42.8	46.5	−3.70	41.7	42.5	−.80	−2.25	47.03
Castillear-1-4-2×Yauco-Torre	31.2	29.70	+1.50	36.50	45.4	43.90	−1.50	32.7	44.85	−12.15	−5.32	36.45
Vicens Flint-2-9-2×Yauco-Torre	39.7	30.22	+9.48	48.00	42.2	41.00	+1.20	37.5	45.15	−7.65	−3.22	41.85
Cacique-1-78-2×Yauco-Torre	34.9	30.1	+4.80	36.15	41.2	41.0	−0.20	41.0	48.80	−7.80	−3.80	38.31
Castillear-1-3-4×Yauco-Torre	26.9	29.5	−2.60	44.10	58.6	41.6	+17.00	40.1	46.20	−6.10	+5.45	42.42
Castillear-1-3-2×Yauco-Torre	26.4	24.3	+2.10	54.00	46.5	44.5	+2.00	47.0	49.50	−2.50	−.25	43.47
Castillear-1-2-1×Yauco-Torre	46.5	26.4	+20.10	36.00	44.3	46.15	−1.85	48.7	50.85	−2.15	−2.00	43.88
Cacique-1-50-2×Yauco-Torre	23.8	22.54	+1.26	21.90	32.3	45.85	−13.55	41.5	52.70	−11.20	−12.37	29.87
Cacique-1-93-1×Yauco-Torre	21.7	18.76	+2.94	19.80	34.8	45.85	−11.05	45.3	52.6	−7.30	−9.17	30.40
Vicens Flint-2-3-1×Yauco-Torre	19.0	17.80	+1.20	37.80	55.2	43.60	+11.60	48.5	52.15	−3.65	+3.98	40.13
Castillear-1-3-3×Yauco-Torre	19.0	16.74	+2.26	40.10	43.3	41.90	+1.40	48.5	55.50	−7.00	−2.80	37.72
Marcucci Droop-10-1-1×Yauco-Torre	27.5	27.48	+.02	39.05	22.2	39.30	−17.10	27.4	52.30	−24.90	−21.00	29.04
Average	36.52	27.56	+8.96	41.97	42.75	45.00	−2.25	39.10	46.97	−7.87	−5.06	--------

Taken as a group the mean increase of the line-variety hybrids over the staminate parent variety in plot E was 8.96 bushels of air-dry shelled corn per acre (table 1). The 27 line-variety hybrids averaged 36.52 bushels per acre, while the Yauco-Torre check variety averaged only 27.56 bushels in an equal number of plots. The odds are 9,999 : 1 that this difference is significant.

The spacing and field conditions in plots K and L were the same as those in plot E. Instead of the Yauco-Torre corn being used as the check variety, Castillear-1-5-1 × Yauco-Torre, one of the hybrids which germinated well and whose inbred parent was outstanding for growth, vigor, and grain production, was chosen. The other 26 hybrids were inferior to those of the check in 38 of 52 comparisons. A general comparison was made between Castillear-1-5-1 × Yauco-Torre and the other 26 hybrids using the average yield of two plots of each variety as a unit. As an average for both plots K and L Castillear-1-5-1 × Yauco-Torre outyielded all the other hybrids by a margin of 5.06 bushels per acre. The odds are 4,999 : 1 that this is a significant difference.

PARALLEL PERFORMANCE IN SECOND-GENERATION SIBS

TABLE 2.—*Yields of air-dry shelled corn per acre from second-generation sib lines outcrossed with Yauco-Torre corn in the Yauco-Torre line-variety trials, Mayaguez, 1927*

First-generation inbred parent	Yields [a] of second-generation sib lines crossed with Yauco-Torre corn								Average shelled corn per acre yield [b] of sib crosses
	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	Shelled corn per acre	Proportion of Yauco-Torre yield	
	Bushels	*Percent*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Bushels*
Castillear 1-5	44.8	162.9	45.99	167.2					45.39±0.59
Castillear-1-3	46.4	168.6	43.47	157.8	42.44	154.1	37.72	137.0	42.51±2.42
Castillear-1-4	34.34	124.8	47.03	171.0	29.09	105.6			36.82±6.81
Vicens Flint-2-9	41.8	151.8	36.9	134.1					39.35±2.45
Cacique-1-2	43.66	158.7	48.99	177.9					46.33±2.67
Cacique-1-50	33.06	120.1	29.87	108.6					31.46±1.59
Yauco-Torre staminate parent	27.56								

[a] Each yield is that of a cross between a second-generation line derived from a first-generation line given in column 1 and Yauco-Torre corn and is the average of 4 plots

[b] Averages of all sib crosses in 8 to 16 plots

Comparison is made in table 2 between yields of six groups of second-generation sib lines crossed with Yauco-Torre corn and those of the Yauco-Torre staminate parent. The first-generation inbred-parent line of each group of sibs is given in column 1. In the case of each line-variety cross the average yield of 4 plots is given; the average yields of the sib groups given in the last column are those of 8 to 16 plots and that of the Yauco-Torre corn is the average of 27 plots. Groupings were made as follows: Very high yielders, the hybrids that exceeded the Yauco-Torre corn by 50 percent or over; high yielders, those that exceeded it by from 25 to 49 percent; and low yielders, those that exceeded it by less than 25 percent. Of 6 different groups of second-generation sibs lines, 5 gave a parallel performance, i.e., they were either all high yielders or all low or medium yielders when outcrossed with an open-pollinated variety.

The lines used in the line-variety crosses were largely derived from the three parent ears Castillear-1, Vicens Flint-2, and Cacique-1. Among 11 hybrids whose pistillate parents were Castillear-1 derivatives there were 8, or nearly three fourths, which were very high yielders, 1 high yielder, and 1 low yielder. Only half of the hybrids whose pistillate parents were derived from the other ears were very high yielders. Castillear-1 is outstanding as a source of desirable lines. This indicates the desirability of concentrating the work of self-pollination on a few high-yielding ear-to-row selections.

PARENT-PROGENY CORRELATION STUDIES, 1927 CROP

Correlation studies (see tabulation on p. 351) were made between line-variety yields and various characters of the inbred pistillate parents including yield, leaf width, seedling foliage weight, percentage of soft starch by weight, and the kernel type as indicated by the degree of denting. The average yield of the 4 plots of each hybrid in comparison with the general average of 31 plots of the Yauco-Torre check corn were used as the basis for the yield comparison. The yields were expressed in percentages of the check. In this way a comparison could be made between yields of the inbred lines in 1925 and 1926, and those of the hybrids in 1927. The seasonal variation from year to year makes a direct comparison in actual yields undependable. Tables 3 and 4 give the data for the years 1925 to 1927, inclusive, on both the inbred pistillate and the corresponding line-variety hybrids.

TABLE 3.—*Parent-progeny study of Yauco-Torre line varieties and inbred parents, 1925–27*

First inbred generation line	Shelled corn per acre: First selfed generation: Yield	Shelled corn per acre: First selfed generation: Proportion of check	Shelled corn per acre: Second selfed generation, August 1926: Yield	Shelled corn per acre: Second selfed generation, August 1926: Proportion of Yauco-Torre corn	Shelled corn per acre: Average yield of first and second inbred generations, proportion of check	Shelled corn per acre: Outcrossed with Yauco-Torre corn, July 1927: Yield	Shelled corn per acre: Outcrossed with Yauco-Torre corn, July 1927: Proportion of Yauco-Torre check	Ears per 100 bearing plants of S_2 line	Green foliage of S_2 parent lines at 3 weeks: Weight	Green foliage of S_2 parent lines at 3 weeks: Proportion of Yauco-Torre check	Dry foliage of hybrids at 3 weeks: Weight	Dry foliage of hybrids at 3 weeks: Proportion of Yauco-Torre check [a]	Weight of 100 kernels: S_2 lines	Weight of 100 kernels: xenia generation	Soft starch by weight
	Bushels	*Percent*	*Bushels*	*Percent*	*Percent*	*Bushels*	*Percent*	*Number*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*
Castillear-1-5-1	40.3	76.8	44.1	90.3	88.0	45.99	167.2	141.8	36.0	130.6	14.02	111.3	32.9	29.3	23.97
Castillear-1-5-2	40.3	76.8	45.7	103.1	89.9	44.8	162.9	142.1	31.5	95.7	13.82	109.8	35.1	28.6	--------
Castillear-1-1-1	51.2	94.3	26.4	59.7	77.0	47.6	173.1	101.8	28.7	91.6	16.59	106.0	37.4	30.5	27.39
Castillear-1-2-1	61.0	110.0	14.7	33.2	71.6	43.88	159.4	128.2	33.0	84.2	13.18	103.6	37.9	24.9	35.23
Castillear-1-3-1	66.5	145.1	24.4	54.9	100.0	46.41	168.6	152.7	44.2	136.6	18.81	143.7	47.3	32.2	22.48
Castillear-1-3-2	66.5	145.1	29.4	66.3	105.7	43.47	158.1	121.8	37.0	114.5	17.83	136.1	32.3	28.6	29.76
Castillear-1-3-3	66.5	145.1	12.1	27.3	86.2	37.72	137.1	126.7	28.9	82.5	14.53	106.8	27.6	23.7	18.90
Castillear-1-3-4	66.5	145.1	14.8	33.4	89.2	42.42	154.1	134.9	26.4	92.2	15.69	115.1	40.3	26.1	22.80
Castillear-1-4-1	43.3	74.7	21.2	47.9	61.3	34.44	124.8	171.3	20.9	66.7	11.30	75.3	27.8	24.0	20.19
Castillear-1-4-2	43.3	74.7	15.4	34.7	54.7	29.09	105.8	136.4	29.7	95.0	16.53	110.2	36.4	28.0	21.00
Castillear-1-4-3	43.3	74.7	17.9	40.3	57.5	47.03	171.0	125.0	37.4	115.6	14.90	112.4	33.3	24.2	21.86
Cacique-1-2-1	47.6	89.2	31.7	71.6	80.4	43.66	158.7	160.0	25.7	79.6	13.12	95.0	33.1	25.6	19.58
Cacique-1-2-4	47.6	89.2	25.3	57.1	73.1	48.99	177.9	134.0	22.9	70.8	14.11	102.1	22.7	28.6	16.86
Cacique-1-50-1	11.6	21.1	12.7	28.5	24.9	33.06	120.1	127.5	28.2	87.4	13.70	104.6	32.5	24.7	19.88
Cacique-1-50-2	11.6	21.1	5.2	11.8	16.4	29.87	108.7	125.5	20.6	63.8	11.02	84.2	30.4	26.4	25.10
Cacique-1-78-2	19.5	39.0	23.4	52.7	45.8	38.31	139.1	159.4	19.7	61.1	14.13	136.5	29.0	30.0	14.62
Cacique-1-67-1	7.3	13.2	27.3	61.7	37.4	41.65	151.4	131.2	20.9	64.2	12.56	103.2	28.8	25.6	26.11
Cacique-1-93-1	2.4	4.6	15.2	34.2	19.4	30.40	110.4	133.3	24.6	76.1	11.78	113.7	25.6	25.3	[illegible]
Vicens Flint-2-9-1	25.4	71.5	26.5	59.9	65.7	36.96	134.5	125.9	29.6	91.7	14.62	106.1	41.0	29.0	23.39
Vicens Flint-2-9-2	25.4	71.5	9.9	22.5	47.0	41.85	152.0	105.9	29.5	91.3	12.45	90.5	30.6	22.4	12.59
Vicens Flint-2-2-2	37.2	88.4	21.2	47.9	68.1	37.65	136.9	115.2	22.4	69.3	13.20	108.5	32.9	29.2	21.40
Vicens Flint-2-4-1	48.2	101.3	21.8	49.0	75.1	42.63	155.0	134.0	28.9	89.4	15.30	148.3	37.5	29.2	16.78
Vicens Flint-2-3-1	9.7	27.6	13.6	30.7	29.1	40.13	145.8	108.0	22.9	70.8	11.51	112.0	36.0	27.9	12.46
Vicens Flint-2-12-1	33.6	91.6	23.9	53.5	72.7	44.10	160.2	108.6	26.1	80.8	13.80	94.8	35.8	28.8	18.02
Vicens Flint-2-14-2	40.8	76.9	18.5	41.7	59.3	33.88	123.1	116.2	27.9	86.4	14.80	101.7	44.5	25.5	16.82
Vicens-21-1-3	48.1	12.9	10.9	24.6	18.8	35.97	130.7	132.6	30.8	95.5	14.76	111.3	38.0	27.7	28.52
Marcucci Droop-10-1-1	29.3	53.3	3.6	7.9	30.6	29.04	105.6	101.8	27.7	88.5	--------	121.0	33.2	--------	--------
Yauco-Torre corn, check	--------	--------	44.3	--------	--------	27.54	--------	--------	[b] 32.3	--------	--------	--------	--------	--------	--------

[a] The dry foliage weights of the Yauco-Torre corn checks were variable in the different germination flats and are not given

[b] This is the average of all checks, the percentages are based on individual check weights.

TABLE 4.—*Plant characters of first and second inbred generations of Yauco-Torre line variety pistillate parents, 1925–27*

Second inbred generation line	Second inbred generation data									Leaf width of broadest leaf				Height to tassel base (30 to 50 plants)				Average ear dimensions of second inbred generation, 40 to 50 plants			
	Ears examined	Ears in each class indicated					Diseased plants	Barren plants	Mode for number of kernel rows	First inbred generation, average for 20 plants at 50 days		Second inbred generation, average for 50 plants at maturity		First inbred generation		Second inbred generation		Total length per plant	Average ear length	Total diameter per plant	Average ear diameter
		Deeply creased dent	Creased dent	Dimpled dent	Shallow dimpled dent	Flint				Width	Proportion of check	Width	Proportion of check	Average height	Proportion of check	Average height	Proportion of check				
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Centimeters*	*Percent*	*Centimeters*	*Percent*	*Decimeters*	*Percent*	*Decimeters*	*Percent*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
Castillear-1-2-1	24	37.5	41.7	8.3	12.5		5.9	7.8	12	9.70	105.2	8.6	80.3	25.02	105.9	17.7	78.5	23.0	18.2	4.76	3.76
Castillear-1-3-1	24		19.2	54.2	12.5	14.1	1.8	0	10-12	8.00	99.2	11.0	104.7	24.79	108.2	19.0	85.2	29.6	19.3	6.61	4.32
Castillear-1-3-2	45	4.4	33.4	46.7	13.3	2.2	1.8	3.6	10-12	8.00	99.2	9.1	86.5	24.79	108.2	17.8	81.3	23.7	19.7	5.10	4.22
Castillear-1-3-3	24		12.5	8.3	45.9	33.3	0	13.5	12	8.00	99.2	8.3	81.5	24.79	108.2	13.7	62.1	16.1	12.8	4.75	3.80
Castillear-1-3-4	28		25.0	42.9	25.0	7.1	0	15.7		8.00	99.2	9.0	86.5	24.79	108.2	16.6	77.7	20.9	15.4	5.40	3.98
Castillear-1-4-1	35	2.8	17.2	25.7	42.9	11.4	0	17.6	12	8.88	110.8	10.1	95.0	24.10	101.4	16.8	79.8	26.2	15.6	6.84	4.06
Castillear-1-4-2	21		47.7	28.6	14.3	9.4	0	4.3		8.88	110.8	10.1	95.0	24.10	101.4	17.3	81.4	15.9	14.9	4.15	3.90
Castillear-1-4-3	31	3.2	64.6	22.8	9.4		0	11.1	10-12	8.88	110.8	9.5	87.3	24.10	101.4	18.9	87.9	19.5	15.6	5.00	4.00
Castillear-1-5-1	40	2.5	75.1	17.4	5.0		1.7	3.5	14	8.86	106.4	10.9	98.2	24.18	99.1	21.3	97.5	26.9	18.8	6.67	4.67
Castillear-1-5-2	37		78.5	21.5			0	0	14	8.86	106.4	11.7	108.6	24.18	99.1	21.5	93.3	27.3	19.3	6.75	4.76
Castillear-1-1-1	45	2.2	53.3	31.2	13.3		0	0	12-14	8.91	118.3	10.0	93.5	24.7	108.3	17.7	79.5	19.1	18.7	4.80	4.72
Vicens Flint-2-9-1	30		10.0	50.0	23.4	16.6	1.8	0	10-12	7.52	98.8	9.2	80.5	22.21	103.7	20.0	87.5	20.4	16.2	5.16	4.12
Vicens Flint-2-9-2	21		4.7	42.8	23.8	28.7	0	7.3	10-12	7.52	98.8	9.2	83.1	22.21	103.7	17.8	78.1	15.8	14.8	3.97	3.74
Vicens Flint-2-2-2	29	10.0	62.4	20.7	6.9		0	19.3	10-12	8.73	106.8	9.2	85.2	25.14	111.8	19.7	89.5	19.9	17.4	5.22	4.55
Vicens Flint-2-3-1	24		4.2		29.2	66.6	2.78	6.3	10-12	7.95	97.0	7.7	71.0	22.56	97.7	14.9	65.1	15.2	14.8	4.34	4.01
Vicens Flint-2-4-2	28		14.2	28.6	43.0	14.2	0	2.2	10	8.41	104.1	8.8	78.4	25.54	107.9	18.0	79.5	22.4	16.7	4.88	3.64
Vicens Flint-2-12-1	40		12.5	37.5	30.0	20.0	0	0	10-12	8.67	106.3	9.6	87.2	22.90	100.8	17.0	76.7	16.9	16.3	4.35	3.98
Vicens Flint-2-14-2	18	5.0	11.1	27.8	27.8	27.8	11.8	8.8	12	9.33	106.6	7.3	67.3	23.20	100.0	16.8	77.0	16.4	14.2	4.87	4.22
Cacique-1-2-1	36		52.8	33.3	13.9		11.5	3.85	12-14	8.94	103.3	9.9	90.5	22.10	94.3	19.6	81.2	25.0	14.9	6.89	4.12
Cacique-1-2-4	24		12.5	29.2	50.0	8.3	1.8	0	14	8.94	103.3	10.2	92.5	22.10	94.3	18.8	79.3	17.4	16.3	4.71	4.40
Cacique-1-50-1	18		33.3	22.2	33.3	11.2	3.6	7.3	12	6.49	84.0	9.6	87.5	21.40	90.8	18.8	84.7	22.0	17.1	4.75	3.70
Cacique-1-50-2	9	11.1	44.5	33.3	11.1		11.7	3.9	12	6.49	84.0	9.0	82.0	21.40	90.8	17.8	79.2	17.3	13.5	4.92	3.84
Cacique-1-67-1	22	4.5	50.0	31.8	13.7		3.5	5.5	14	6.22	69.3	10.7	97.0	19.40	82.2	17.3	75.6	18.3	13.9	5.48	4.00
Cacique-1-78-2	41		9.8	22.0	51.2	17.0	2.9	0	10-12	6.81	79.9	9.8	88.3	22.07	95.4	18.9	84.1	23.7	18.4	5.12	3.98
Cacique-1-93-1	25			8.0	32.0	60.0	6.6	3.3	12	6.65	67.7	8.6	79.7	17.90	80.7	13.5	63.2	19.1	14.0	4.96	3.64
Marcucci Droop-10-1-1	11		88.9	11.1			0	10.5	12	8.02	93.9	9.9	88.3	21.60	105.9	21.0	92.9	23.3	22.1	4.83	3.58
Vicens-21-1-3	14	28.6	50.0	21.4			2.2	13.1	12	8.78	99.8	10.3	94.7	24.10	101.3	22.1	99.3	21.4	15.8	5.82	4.32
Yauco-Torre check												10.9				22.2		28.0	19.9	6.26	4.47

RELATION BETWEEN LINE-VARIETY YIELD AND PERCENTAGE OF SOFT STARCH OF THE INBRED PISTILLATE PARENT

The percentage of soft starch was determined by dissecting the soft starch from the halves of 50 kernels, 2 kernels being taken from near the center of each of 25 different ears of a given inbred line. Weights of dissected material were taken after the material had dried in an electric oven for 2 hours at 108° C. There was no marked relation between the yields of the line-variety hybrids and the proportion of soft starch in the inbred pistillate parent. The coefficient of correlation was low, only 0.198 ± 0.129. The 10 lines derived from parent ear Castillear-1 averaged 24.34 percent of soft starch and were generally superior in yield when outcrossed to the 7 lines derived from Cacique-1 which averaged 19.36 percent, and to the 7 lines from ear Vicens Flint-2 which averaged 17.34 percent.

RELATION BETWEEN LINE-VARIETY YIELDS AND MODE FOR DEGREE OF DENTING

The progeny ears of each second-generation line were classified according to kernel types into five groups—deeply creased dent, creased dent, dimpled dent, shallow dimpled dent, and flint. Where fewer than 20 ears were classified no more were available due to barrenness, or disease, or weathering in the field. The correlation was positive ($r = 0.377 \pm 0.111$), but not high enough to justify a rigid elimination of flinty types of inbred lines.

RELATION BETWEEN LINE-VARIETY YIELDS AND PERCENTAGE OF BARREN AND DISEASED PLANTS

The total percentage of barren and diseased plants of each inbred parent was negatively correlated with yield of the line varieties ($r = -0.441 \pm 0.025$). Of the 12 hybrids below average in yield, 9 had inbred parents with more than 10 percent of diseased and barren plants. Of the 7 highest yielding hybrids, 5 had inbred parents with less than 4 percent of diseased and barren plants, and only 1 had an inbred parent with as much as 10 percent.

LINE-VARIETY YIELDS AND GERMINATION

The germination vigor is expressed as the foliage weight of 20 seedlings when 21 days old in percentage of that of Yauco-Torre seedlings grown in the same germination flat. The marked increase in this factor due to outcrossing with open-pollinated corn is shown in table 3. The inbred parents were, with two exceptions, inferior in seedling size to the Yauco-Torre corn, whereas the hybrids were generally superior, and on this basis the resulting generally superior yields could have been predicted. The average seedling weight superiority of hybrid over the open-pollinated staminate parent was 10.1 percent, and only 5 of 27 hybrids were inferior. The superior seedling vigor of most of the 27 hybrids is rather remarkable considering that the weight per 100 kernels of the Yauco-Torre pistillate parent was 37.5 g, whereas none of the hybrids exceeded 32.2 g in the xenia generation.

Considered within related groups, the line-variety hybrid with the most rapid early growth was the highest yielder; this was true in 4 groups of sib hybrids, not true in 1, and doubtful in 1 group. The correlation coefficient between the 26 line-variety yields and the hybrid-seedling foliage weight was only 0.283 ± 0.124 and did not confirm the observations made on sib groups. This rather low correlation indicates that it is not safe to discard any but the very poorest germinators in the line-variety crosses.

YIELDS OF THE LINE-VARIETY HYBRIDS IN RELATION TO LEAF WIDTH AND HEIGHT OF THE INBRED PARENT

Among the six groups of related line-variety hybrids the broadest leafed line within each group in the second inbred generation produced the highest yielding hybrid. There was one exception, that of the Castillear-1-4 derivatives. The correlation between leaf width of the inbred parent and line-variety yields ($r = 0.315 \pm 0.117$) was not so high as that for parent-progeny yields but tends to confirm observations made on groups of sib hybrids. There was no such relationship apparent between yield and height, there being equally as many low- as high-yielding sib hybrids with tall pistillate parents.

LINE-VARIETY YIELDS AND YIELDS OF FIRST TWO INBRED GENERATIONS

A high and significant correlation was found between the line-variety yields and the average yields of the inbred parent in the first two inbred generations ($r = 0.638 \pm 0.0767$). Of the various characters studied yield is apparently the most dependable basis for elimination in inbred lines. All except 1 of the 9 lines that yielded 76 percent as much as the open-pollinated check variety in the first and second inbred generations, outyielded the check by 50 percent in line-variety combinations. Among 7 lines that were inferior to the check by 50 percent in the first 2 inbred generations, there were in the corresponding line-variety hybrids 3 low yielders, 2 high yielders, and 2 very high yielders. The elimination of all lines except those that averaged 76 percent of the check in the first 2 inbred generations would have retained 8 of 14 lines which produced very high yielding line-variety hybrids.

LINE-RECESSIVE YIELD TRIALS, MAYAGUEZ, 1928

Line-recessive yield trials were made at Mayaguez in 1928 to determine whether high-yielding hybrids could be produced by crossing lines inbred two or three generations and whether lines with larger plant growth and longer ears would produce higher yields when crossed with an unrelated, white line assumed as recessive because of white kernel color and inferiority in plant size, ear size, and yield. The spacing and size of plots were the same as those used in the 1927 line-variety yield trials. Growing conditions were good, and no abnormal weather interfered with grain production.

The material used in the 1928 yield trials consisted of hybrids between a white-kerneled line selfed three generations and 24 yellow-kerneled lines selfed two generations. The yellow lines were derived from six unrelated ears from corn districts near Aibonito, Barranquitas, Coamo, Lares, Morovis, and Peñuelas. With the exception of the ear from Peñuelas, the parent ears were selected on the basis of high total ear longisection area per plant and were entirely unrelated to the lines used in the 1927 line-variety yield trials.

The Peñuelas lines were derived from Castillear-1, the ear which was outstanding in the 1927 trials as a source of lines that gave high yield in line-variety combinations. The progeny of this ear was open-pollinated during one season, and in the following generation 100 selfed lines were secured. From these 15 lines were retained which outgrew Yauco-Torre corn at 3 weeks. The first generation lines, nos. 21 and 50, were outstanding for healthy foliage and large plant growth in field trials, and from these the Peñuelas lines were derived.

The white line, Cacique-1-2-4-6 used as the staminate parent, makes a very inferior plant growth and produces small ears, 10–13 cm long and very small kernels. It yields between 8 and 10 bushels of shelled corn per acre, or about one fourth normal, and decidedly less than all the yellow-kerneled lines. Because of its decided inferiority, the white line was thought to afford a good test for the potential yielding values of the yellow lines, as only the more vigorous yellow types could be expected to give a high yield in F_1 combination. These hybrids were considered as contrasting with the line-variety hybrids in which the variety is the source of many dominant characters and an infinite variety of combinations is the result. In the former type of hybrid the yellow lines, being larger in ears, kernels, and plant growth than the staminate parent, constitute the principal source of dominant characters; the variety of combinations that result is not so great as in line-variety hybrids for it depends largely on the variability in the yellow lines.

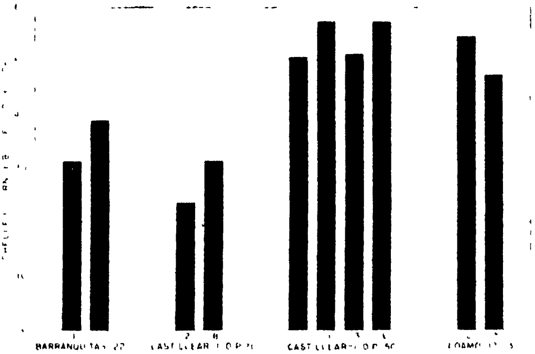

FIGURE 1.—Parallel performance in second-generation sibs. Mayaguez. 1928. The bars represent yields in bushels of shelled corn per acre of four groups of sib second-generation corn lines which have all been outcrossed with the same white line. Related hybrids were either all high yielding or all low yielding.

PARALLEL PERFORMANCE IN SECOND-GENERATION SIBS

Figure 1 shows diagrammatically the parallel performance of four different groups of sibs in hybrid combinations with the common white pollinator. The bars represent the average yields in bushels per acre. The bars representing yields of related hybrids are grouped together, and underneath in each case is given the first inbred generation line from which they were derived on the pistillate side. There was parallel performance in all four related groups; hybrids derived from Barranquitas-22-11 and Castillear-1-o.p.-21 were all low yielders while those from Coamo-12-13 and Castillear-1-o.p.-50 were all high yielders.

The superior crossing values of the four second-generation sib lines derived from Castillear-1-o.p.-50 are demonstrated when their hybrids are compared as a group with the Yauco-Torre corn. The eight plots outyielded the latter by an average of 15.51 bushels per acre. The odds are 1,732:1 that this difference is not entirely due to chance. Not only was the yield significantly greater but also the kernels had a brighter luster and the grain quality was superior, due to less decay at the tip ends of the ears. The parallel performance of these four groups of sibs when crossed with a nonrelated white recessive variety indicates that within the limited breeding material used it was feasible to select lines with high crossing values in the second inbred generation.

TABLE 5.—*Parent-progeny study of line-recessive*[a] *crosses and second-generation dominant parent lines, 1928*

Second-generation line parent	September 1927 data on second-generation lines					1928 data on line-recessive crosses													
							Average of 30 to 35 plants			Yield of air-dry shelled corn per acre						Yield of selection, average of plots K and L		Average height to tassel base (18 to 20 plants)	
										Plot K			Plot L						
	Average height to tassel base (30 plants)	Ears per 100 bearing plants	Total ear length per plant	Diameter of larger ears	Total longisection area[b]	Ears per 100 bearing plants	Total ear diameter	Total ear length	Total longisection area[b] of ears per plant	Selection	Average nearest 3 checks[c] plots	Yield of selection as proportion of nearest 3 check plots	Selection	Check	Selection as proportion of check	Yield	Yield of selection as proportion of nearest 3 check plots	Hybrids	Yauco-Torre check
	Centimeters	*Number*	*Centimeters*	*Centimeters*	*Centimeters^2*	*Number*	*Centimeters*	*Centimeters*	*Centimeters^2*	*Bushels*	*Bushels*	*Percent*	*Bushels*	*Bushels*	*Percent*	*Bushels*	*Percent*	*Decimeters*	*Decimeters*
Aibonito-4-5-3	121.9	133	16.8	3.14	52.1	114	4.96	18.07	89.6	35.3	34.4	102.7	33.7	44.83	75.2	34.5	88.6	18.2	19.2
Aibonito-4-8-4	136.1	143	16.8	3.53	59.4	106	4.74	17.26	81.9	38.4	34.4	111.6	41.2	44.83	92.1	39.8	101.8	19.1	19.5
Aibonito-4-12-1	160.9	174	23.4	3.50	81.9	109	5.06	19.36	98.0	45.4	37.6	120.8	39.1	43.30	90.2	42.25	105.5	19.3	20.1
Aibonito-4-15-1	128.1	162	19.7	3.55	70.0	111	4.83	18.32	88.5	34.8	34.7	100.2	42.1	37.20	113.1	38.45	106.6	18.4	20.5
Barranquitas-22-9-1	136.4	160	23.2	3.31	76.7	123	5.29	20.63	109.2	21.2	34.05	62.3	20.6	33.40	61.7	20.9	62.0	17.9	20.0
Barranquitas-22-11-1	177.4	156	20.8	2.76	57.4	130	5.66	20.54	116.1	20.4	33.43	61.1	40.6	34.4	118.0	30.5	89.5	19.4	18.3
Barranquitas-22-11-2	185.6	100	13.0	3.40	44.2	122	5.47	20.02	109.6	34.5	31.70	108.8	44.0	36.33	121.0	39.25	114.9	19.9	18.7
Barranquitas-22-15-1	141.7	169	23.8	2.77	66.0	124	5.40	21.40	115.6	34.6	33.40	103.4	39.3	36.90	106.4	36.95	104.9	19.8	19.7
Coamo-12-1-1	121.9	109	17.1	3.36	57.6	100	4.54	17.81	81.2	38.3	41.10	93.1	34.9	33.53	104.2	36.6	98.6	17.2	19.3
Coamo-12-4-3	126.7	174	23.2	3.05	70.7	123	6.02	20.80	125.0	56.8	46.43	122.5	39.2	37.30	105.2	48.1	113.8	18.2	18.1
Coamo-12-13-2	141.8	170	21.9	3.47	76.0	121	5.32	20.85	110.9	53.8	47.90	112.2	53.9	38.97	138.4	53.85	125.3	19.4	19.5
Coamo-12-13-5	120.9	165	20.2	3.09	62.5	133	5.91	20.77	122.7	51.0	47.90	106.4	42.3	38.9	108.7	46.65	107.5	17.6	19.0
Lares-6-4-2	142.5	138	17.6	3.05	53.7	109	4.65	20.07	93.3	42.3	46.50	91.0	33.2	39.63	83.7	37.75	87.3	18.1	19.5
Lares-6-7-2	168.4	166	21.8	3.56	77.6	117	5.37	19.76	106.2	47.3	46.50	101.7	36.0	39.63	90.8	41.65	96.2	21.0	19.8
Morovis-6-2-3	139.8	135	20.5	2.59	53.1	103	4.91	19.95	98.0	30.2	48.70	62.0	50.5	38.13	132.3	40.35	97.1	17.5	19.6
Morovis-6-6-1	141.0	169	21.0	4.11	86.3	139	7.54	25.15	189.8	70.5	53.40	132.0	58.3	39.1	149.1	64.4	140.5	18.5	20.4
Morovis-6-7-4	126.7	142	14.5	3.12	45.2	116	5.35	20.23	108.2	39.9	52.63	75.7	39.2	39.9	98.2	39.55	86.9	17.7	20.6
Castillear-1-3-o.p.-7-2	116.1	145	17.1	3.04	51.8	115	4.69	17.42	81.9	32.9	38.6	85.3	29.0	40.8	71.0	30.95	78.1	16.2	18.1
Castillear-1-o.p.-21-2	96.1	132	15.5	3.58	55.5	103	4.83	18.53	89.5	20.6	45.73	45.1	25.1	40.6	61.7	22.85	53.4	17.2	18.2
Castillear-1-o.p.-21-8	116.6	139	17.0	4.14	70.4	123	5.65	16.64	94.0	24.9	48.80	51.0	38.0	40.63	93.4	31.45	72.2	16.4	19.0
Castillear-1-o.p.-50-1	158.1	147	23.0	3.87	89.0	133	6.06	21.76	132.0	63.5	44.77	142.0	51.2	40.73	125.7	57.35	133.8	18.8	20.2
Castillear-1-o.p.-50-2	174.5	168	20.6	3.69	76.0	113	5.09	19.47	99.1	61.9	40.13	154.1	38.3	36.27	105.8	50.1	129.9	19.6	20.3
Castillear-1-o.p.-50-3	153.2	164	21.2	3.69	78.3	144	6.23	23.23	144.8	62.3	36.06	173.0	40.3	34.60	116.6	51.3	144.8	20.7	19.8
Castillear-1-o.p.-50-6	164.2	182	21.5	3.80	81.9	151	6.19	23.43	144.8	56.8	37.93	149.8	57.0	36.70	155.0	56.9	152.4	20.2	19.2

[a] Common pollinator = Castillear-1-2-4-6, a white recessive, inbred 3 times, which produces 10 bushels of shelled corn per acre, has a total ear length per plant of 13 centimeters, and a height of 11.1 decimeters to tassel base.

[b] Diameters of larger ears only were used to calculate total longisection area per plant, as incomplete pollination prevented the determination of the diameters of the other ears of the inbred parents.

[c] Yauco-Torre = corn check variety.

As may be observed in the data on height in table 5 the growth of the check plants which grew in rows adjoining the hybrids was not markedly affected by the plant size of the latter. As a rule, the check grew just as tall next to the taller hybrids as it did in the rest of the field. The competition between varieties does not appear to have been marked, and there were several instances of high yields of hybrids which were quite inferior to the check in height, i.e., those of Morovis-6-6-1 and Coamo-12-13-2.

PARENT-PROGENY CORRELATION STUDIES, 1928 CROP

Since the white staminate parent was almost homozygous for kernel size, color, ear length, and height, it was predicted that most of the variability in the hybrids would be due to the influence of the yellow parents. It was therefore thought that high correlations in plant height and yield would be secured between the line-recessive crosses and inbred yellow parents. Unfortunately poor pollination due to violent storms in 1927 prevented the securing of data on yield of parent lines. There was, however, a high correlation for longisection area of ears per plant between line-recessive hybrids and the respective yellow-line parents ($r=0.549\pm0.096$), and as was predicted, the parent-progeny correlation for height was high ($r=0.834\pm0.043$). Since hybrid yields were also strongly correlated with these characters of the inbred parents, $r=0.595\pm0.089$ for yield and height and $r=0.540\pm0.097$ for yield and total ear longisection area per plant, it follows that they could have been used as a basis for elimination in the second inbred generation. These results tend to corroborate those secured by Jenkins[9] on lines inbred 3 or 4 generations.

The correlations between line-recessive hybrid yields and ear length or ear diameter of inbred parents were nearly double those for 1927 line-variety yields and the same characters of the inbred parents, as shown below. Apparently much less variability in hybrid yield and hybrid plant growth resulted from the use of the open-pollinated variety than through the use of the recessive line. As a test for relative dominance among inbred lines the line-recessive method appears to be more effective. In the line-variety hybrids the dominant characters contributed by the open-pollinated variety tended to obscure those of the selfed lines.

Characters correlated with line-variety yields:	*Coefficients of correlation*
Average yield of first and second inbred generations	0.638 ± 0.0767
Seedling foliage weight of hybrids at 3 weeks	.283 ± .124
Mode for degree of denting of inbred parent	.377 ± .111
Percentage of soft starch of inbred parent	.198 ± .129
Total ear length per plant of inbred parent	.189 ± .125
Total ear diameter per plant of inbred parent	.175 ± .125
Leaf width of inbred parent	.315 ± .117
Percent of diseased and barren plants of inbred parent	−441 ± .025
Characters of dominant inbred parents correlated with line-recessive yields:	
Height to tassel base	.549 ± .095
Total ear longisection area per plant	.540 ± .097
Total ear length per plant	.384 ± .117
Total ear diameter per plant	.386 ± .117
Parent-progeny characters correlated:	
Total longisection area of ears per plant of line-recessive hybrids and the same of dominant yellow inbred parents	.549 ± .09[illegible]
Height to tassel base of line-recessive hybrids and the same of dominant inbred parents	.834 ± .043

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[9] JENKINS, M. T. See footnote 7.

The average yield increase over Yauco-Torre check corn was nearly 10 times as great for the 26 line varieties as for the 24 line-recessive hybrids. The average inferiority of the line recessives is apparently owing to the high proportion of lines which were not outstanding for dominant characters and were inferior for plant growth. The yields of the more promising of the line-recessive hybrids, however, compared quite well with those of the high-yielding line varieties.

UTUADO LINE-VARIETY YIELD TRIALS [10]

Line-variety yield trials were made at Utuado to compare yields of line-variety hybrids with those of their pistillate variety parents and with the corn produced on the farm of Tomás García.

Three line-variety hybrids produced at the Isabela substation and five produced through the cooperation of Central Coloso near Isabela were compared with corn locally produced on the García farm and with the pistillate parent varieties. The staminate parents were Castillear-1-5-1 composite and Castillear-1-5-2 composite, derived from the same first generation line; after 4 to 5 generations of self-pollination 6 and 8 sibs, respectively, were lumped. The parent lines of both staminate parents had given high yields in line-variety crosses in 1927. The pistillate parents consisted of corn varieties collected in various parts of the island. In each instance corn was chosen from farms where the practice had been for seed corn to be saved from year to year. The objective was to find distinct types of corn. With the exception of the Aguadilla corn, however, which had dark-orange seed color, red cobs, and was fairly uniform for dimpled to smooth dent kernels and long tapering ears, all the other varieties were similar in type, i.e., a smooth dent with ears of medium length varying in kernel color from light yellow to orange. The corn produced on the García farm came originally from Yauco and was very similar to the Yauco-Torre corn.

The corn from the García farm was used as a check and was planted in every third plot. Hills were spaced 3 feet apart each way, and the corn was thinned to two plants per hill. The plots were 2 rows wide and 208 feet long. Three plots, one thirty-fifth of an acre in area, were planted to each hybrid. The soil was a loose sandy loam, typical of the cornland of the Utuado district. The field was well drained and not under irrigation. A heavy rain that fell a few days after planting washed out the seeds and made it necessary to replant the entire field. Owing in part to adverse conditions at the start, and in part to a steep somewhat stony slope at one end of the field, yields were very erratic.

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[10] Cooperation with the Insular Department of Agriculture.

TABLE 6.—*Yields*[a] *per acre of air-dry shelled corn in the Utuado line-variety-hybrid trials, the yields of hybrids being compared with those of pistillate parents*

Plot no	Hybrid	Yield of hybrids	Yields in adjoining plots of pistillate parents[a]	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
		Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
9	Juarbe-1	29.1	27.7	+1.4	−1.53	2.341
30	Juarbe-1	17.8	21.5	−3.7	−6.63	43.957
51	Juarbe-1	13.1	15.2	−2.1	−5.03	25.301
12	San Sebastián-1	26.1	22.9	+3.2	+.27	.073
33	San Sebastián-1	22.2	20.1	+2.1	−.83	.689
54	San Sebastián-1	12.9	13.2	−.3	−3.23	10.433
15	Peñuelas-1	26.8	17.6	+9.2	+6.27	39.313
36	Peñuelas-1	24.6	17.5	+7.1	+4.17	17.389
18	Coamo-1	25.7	20.5	+5.2	+2.27	5.153
60	Coamo-1	14.2	15.7	−1.5	−4.43	19.625
21	Barranquitas-1	24.0	15.3	+8.7	+5.77	33.293
42	Barranquitas-1	12.7	11.4	+1.3	−1.63	2.657
63	Barranquitas-1	16.8	9.3	+7.5	+4.57	20.885
	Mean			2.93		

[a] N=13 S D =4.123 Z=0.71 Odds=654:1

[b] In making these calculations the differences between the pistillate parents were disregarded, and the yields of the 13 hybrid plots were treated as though they were repetitions of the same cross Each hybrid plot adjoined a plot of its pistillate parent making direct comparisons fair All hybrids listed here had the same staminate parent, Castille n-1-5 1 composite

TABLE 7 —*Yields per acre of air-dry shelled corn in the variety trial, Utuado, P.R.*[a]

Variety	Plot no	Yield based on field weight	Interpolated yields of checks	Plot no.	Yield based on field weight	Interpolated yields of checks	Plot no	Yield based on field weight	Interpolated yields of checks	Average increase or decrease as compared with check (3 plots)	Odds indicating significance of difference
		Bushels	*Bushels*		*Bushels*	*Bushels*		*Bushels*	*Bushels*	*Bushels*	
García	1	19.7									
Aguadilla-2	2	31.8	21.00	23	18.6	23.76	44	14.5	10.53	+3.203	2.53:1
Peñuelas-2	3	28.8	22.30	24	23.8	20.23	45	9.6	12.16	+2.50	3.45:1
García	4	23.6		25	16.7		46	13.8			
Juarbe-2	5	35.8	23.60	26	22.4	16.83	47	13.3	12.34	+6.270	9.16:1
Aguadilla-1	6	26.6	23.60	27	20.9	16.96	48	15.5	11.07	+3.79	166:1
García	7	23.6		28	17.1		49	9.7			
Juarbe	8	27.7	22.64	29	21.5	16.60	50	15.2	11.77	+4.163	262:1
Juarbe-1	9	29.1	21.67	30	17.8	16.10	51	13.1	13.83	+2.80	4.4:1
García	10	20.7		31	15.6		52	15.9			
San Sebastián	11	22.9	20.30	32	20.1	17.10	53	13.2	15.04	+1.253	3.28:1
San Sebastián-1	12	26.1	19.90	33	22.2	18.60	54	12.9	14.17	+2.843	4.96:1
García	13	19.5		34	20.1		55	13.3			
Peñuelas	14	17.6	19.80	35	17.5	19.16	56	11.4	13.0	−1.82	67.3:1
Peñuelas-1	15	26.8	20.10	36	24.6	18.23	57	([b])		+6.535	377:1
García	16	20.4		37	17.3		58	([b])			
Coamo	17	20.5	21.13	38	15.7	15.73	59	15.7	12.1	+.98	1.9:1
Coamo-1	18	25.7	21.86	39	([b])		60	14.2	11.8	+3.12	8.03:1
García	19	22.6		40	12.6		61	11.5			
Barranquitas	20	15.3	24.17	41	11.4	11.36	62	9.3	12.2	−3.91	6.42:1
Barranquitas-1	21	24.0	25.74	42	12.7	10.13	63	16.8	12.9	+1.577	4.26:1
García	22	27.3		43	8.9		64	13.6			

[a] Hybrids have number suffixed to name of pistillate parent. Plot size=one thirty-fifth of an acre consisting of 2 rows, 3 feet apart and 208 feet long Hills were spaced 3 by 3 feet Corn plants were thinned down to 2 plants per hill. All plots were parallel to each other and fronted on the same side of the field.

[b] Omitted on account of incomplete harvest.

No significant differences were found between the yields of individual line-variety hybrids and their respective pistillate parents, which grew in each case in adjoining plots (table 6). It is probable that a larger number of replications would have demonstrated a superiority for some of the individual hybrids as, taken as a group, the 13 plots of hybrids pollinated by Castillear-1-5-1 averaged 2.93 bushels per acre more than the pistillate parents. The 13 hybrid plots were treated as though they were repetitions of the same cross. The odds were 654:1 that this difference was not due to chance. The weights of air-dry shelled corn were based on a shrinkage sample of 100 ears collected, half from corn grown on the García farm and half the interplanted varieties; the moisture content was assumed to be the same for all varieties, as differences in type of corn or degree of maturity were not marked.

TABLE 8.—*Yields*[a] *per acre of air-dry shelled corn in the Utuado line-variety trials the yields of hybrids being compared with those of the García check corn*

[Common pollinator or staminate parent=Castillear-1-5-1 composite, harvested July 28, 1931]

Hybrid	Yield of hybrids	Interpolated yields of García check corn	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
Juarbe-1	29.1	21.67	+7.43	+4.159	17.297
	17.8	16.10	+1.70	−1.571	2.468
	13.1	13.83	−.73	−4.001	16.008
Peñuelas-1	26.8	20.10	+6.70	+3.429	11.758
	24.6	18.23	+6.37	+3.099	9.604
San Sebastián-1	26.1	19.90	+6.20	+2.929	8.579
	22.2	18.6	+3.60	+.329	.108
	12.9	14.17	−1.27	−4.541	20.621
Coamo-1	25.7	21.86	+3.84	+.569	324
	14.2	11.80	+2.40	−.871	.759
Barranquitas-1	24.0	25.74	−1.74	−5.011	25.110
	12.7	10.13	+2.57	−.701	491
	16.8	12.90	+3.90	+.629	396
Aguadilla-1	26.6	23.60	+3.00	−.271	073
	20.9	16.96	+3.94	+.669	448
	15.5	11.07	+4.43	+1.159	1.343
Mean			+3.271		

[a] $N=16$, $S.D.=2.686$; $Z=1.22$. Odds=4,991:1

[b] In making these calculations the differences between the pistillate parents were disregarded, and yields of the 16 hybrid plots were treated as though they were repetitions of the same cross

TABLE 9.—*Yields*[a] *per acre of air-dry shelled corn in the Utuado line-variety trials, the yields of hybrids being compared with those of the García check corn*

[Common pollinator or staminate parent=Castillear-1-5-2 composite]

Hybrid	Yield of hybrids	Interpolated yields of García corn check	Increase or decrease	Deviation from mean, $D-M$ [b]	$(D-M)^2$ [b]
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*
Aguadilla-2	31.8	21.0	+10.8	+6.82	46.512
	18.6	23.76	−5.16	−9.14	83.540
	14.5	10.53	+3.97	−.01	.000
Peñuelas-2	28.8	22.3	+6.50	+2.52	6.350
	23.8	20.23	+3.57	−.41	.168
	9.6	12.16	−2.56	−6.54	42.772
Juarbe-2	35.8	23.6	+12.20	+8.22	67.568
	22.4	16.83	+5.57	+1.59	2.528
	13.3	12.34	+.96	−3.02	9.120
Mean			+3.98		

[a] $N=9$; $S.D.=5.363$; $Z=-0.74$; odds=27:1.

[b] In making the calculations, differences between the pistillate parent varieties from Aguadilla, Peñuelas, and the Juarbe farm were disregarded, and the 9 plot yields were treated as though they were repetitions of the same cross.

As shown in table 7 there were significant differences between the García check corn and two of the hybrids, Peñuelas-1 and Aguadilla-1. The former averaged 3.79 bushels more of the shelled corn per acre than the check and the latter was superior by 6.52 bushels. In the case of the Castillear-1-5-1 hybrids the results are more conclusive if a general comparison is made between the two groups of hybrids and the García corn. The 16 plots of hybrids pollinated by Castillear-1-5-1 composite averaged 3.271 bushels per acre more than the check corn (table 8). The odds are 4,999:1 that this difference is not due to chance. The nine plots of hybrids pollinated by Castillear-1-5-2 composite averaged 3.98 bushels per acre more than the check (table 9). The odds are 27:1 that this difference is not due to chance.

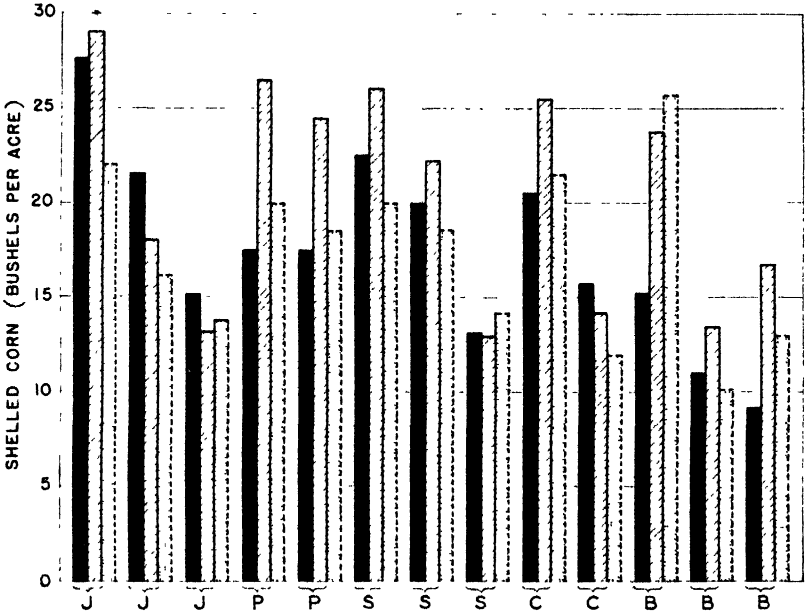

FIGURE 2.—Yields of line-variety hybrids and varieties at Utuado, April 1931. The black bars represent yields of varieties, the hatched bars those of the line-variety hybrids, and the dotted bars those of the García check corn. J=Juarbe, P=Peñuelas, S=San Sebastián, C=Coamo, and B=Barranquitas.

The general superiority of the Castillear-1-5-1 line-variety hybrids is shown graphically in figure 2, and the places of origin of the open-pollinated varieties is indicated in the legend. Each group of bars represents the yields of three adjoining 1/35-acre plots of a variety, and that of a cross between that variety and the inbred line, Castillear-1-5-1, and that of García check corn. The Juarbe pistillate parent was superior to its hybrid with Castillear-1-5-1 and compared well with all of the hybrids in grain production.

DISCUSSION OF RESULTS

The general superiority of the yields of the 27 line-variety crosses to those of the variety parent indicated that this use of second-generation lines would prove effective in increasing yields of corn grown near Yauco.

There were, among both 1927 and 1928 crosses, 10 sib groups, 9 of which gave a parallel performance for yield. This affords some evidence that the parent lines inbred only two generations were approaching uniformity for characters that make for yield in crossbred combination.

A high parent-progeny correlation for yield was secured in 1927, and a still higher one for hybrid yield and total longisection area of ears of inbred parent was secured in 1928. It seems that within the material studied the second-generation lines with the highest crossing values could have been selected prior to crossing on the basis of yield or characters directly associated with yield.

Complete restoration of hybrid vigor resulted from crossing the second-generation lines with a nonrelated open-pollinated variety. Difference in degree of expression of a character contributed by inbred lines tended therefore to be obscured in line-variety combination. Among line recessives there were very high parent-progeny correlations for both height and ear longisection area, and the variability in the germ plasm contributed by the more vigorous yellow-kerneled parents was not apparently affected by the contribution of the so-called recessive parent. Presumably the prepotency of second-generation lines as regards a particular character could be studied to better advantage if they were outcrossed with a line known to be recessive for that character than if crossed with an open-pollinated variety.

SUMMARY AND CONCLUSIONS

Ears were selected from Lajas, Peñuelas, and Jayuya in 1923 and tested for seedling vigor. The ears which were superior in seedling size and drought resistance were placed in ear-to-row yield trials.

Lines were inbred two generations from the three highest yielding parent ears from the three districts and compared in yield and plant characters. They were then crossed with Yauco-Torre, an unrelated open-pollinated variety. In 1927 yield trials were conducted at Mayaguez. The 27 line-variety hybrids taken as a group were significantly superior in yield to the Yauco-Torre corn. Castillear-1-5-1×Yauco-Torre was significantly superior to the other hybrids.

In 5 of 6 groups of sib line-variety hybrids a parallel performance was observed; they were either all high yielding or low yielding.

Correlations were determined between line-variety yields and various characters of inbred parents. The correlation was significant and positive for yield and average yield of first and second inbred generations. The correlation was negative and fairly high, but not significant, for hybrid yield and percentage of barren and diseased plants. Correlation of hybrid yield with mode for degree of denting of kernel, and leaf width of inbred parent were positive and fairly high but not significant. Correlations of hybrid yield with inbred parent characters, percentage of soft starch, and seedling foliage weight were positive but very low. These correlations indicated that within the material studied the average yield of the first and second inbred generations was the most dependable basis for elimination.

Five parent ears from five different districts were selected for high total longisection area of ears per plant and self-pollinated. In 1928 yield trials were conducted at Mayaguez comparing hybrids between second inbred generation derivatives of these ears and a line, assumed as recessive, which had been selfed three times. The line-recessive hybrid yields were not as superior to the Yauco-Torre check corn as were those of the line-variety hybrids. Parallel performance was noted among hybrids secured from four groups of second-generation sibs. Significant correlations were secured between line-recessive hybrid yields, and plant height and total longisection area of ears per plant of the yellow-kerneled inbred lines. Both of these characters of the yellow-kerneled lines appeared dependable as a basis for elimination in the second inbred generation.

Yields significantly superior to Yauco-Torre corn were secured from four sib lines derived from Castillear-1-o.p.-50 by outcrossing them in the second inbred generation with a third-generation line.

In 1931 line-variety crosses between the composites, Castillear-1-5-1 or Castillear-1-5-2 and a number of native varieties outyielded the corn grown on the García farm in the Utuado district. These lines gave superior yields in the second inbred generation outcrossed with the open-pollinated native field corn and continued to do so after the fourth to fifth inbred generation. The margin of superiority does not appear to have been increased by additional generations of inbreeding prior to crossing.

HYPERPARASITISM IN THE CASE OF SOME INTRODUCED LEPIDOPTEROUS TREE DEFOLIATORS[1]

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INTRODUCTION

It has long been known that hyperparasites have a considerable effect on the abundance of primary parasites. In recent years, as more interest has been centered upon the biological control of injurious insects, the problem of the interrelations of parasites, not only between the various primary parasites themselves but also between the primary parasites and the hyperparasites, has called for particular study. It has been considered that one of the chief causes of the reduction in numbers of the parasites imported into New England to aid in the control of the gypsy moth, the brown-tail moth, and other moths has been the activities of hyperparasites. In this paper it is proposed to make available the present information on (1) the extent to which these imported parasites are attacked by secondary parasites of the lepidopterous hosts, (2) the species concerned, and (3) the relative importance of each.

To find out the extent of hyperparasitism in the case of the gypsy moth (*Porthetria dispar* L.), the brown-tail moth (*Nygmia phaeorrhoea* Don.), the satin moth (*Stilpnotia salicis* L.), and the oriental moth (*Cnidocampa flavescens* Walk.) collections of cocoons or puparia of the parasites of these moths were made at various points in their respective infested areas of New England during a period of 4 years, from 1929 to 1932, inclusive.

There was some difficulty in obtaining collections of most of the species of primary parasites in the field. It was thought desirable to make collections at the same localities over a series of years, and as a result many collections were small, especially where the host population was small during the entire period.

Special efforts were made to ascertain the maximum hyperparasitization by collecting cocoons or puparia as near the time of issuance of the primary parasite as possible. In many cases some of the primary parasites had already issued, and these were taken into account with the others. No matter when the collections were made, there were a few cocoons or puparia that had been subjected to the attack of

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[2] The writer is indebted to C. W. Collins, in charge of the laboratory of the Bureau of Entomology, U. S. Department of Agriculture, at Melrose Highlands, Mass., and to C. F. W. Muesebeck, of the Division of Identification and Classification of Insects, Bureau of Entomology, for much helpful advice in the course of the work; to T. H. Jones for permission to use his notes on *Sturmia scutellata*; and, in addition, many thanks are due to R. A. Cushman, C. F. W. Muesebeck, D. L. Parker, and R. T. Webber, of the Bureau of Entomology, for determinations of species.

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secondaries for only a short time, and the inclusion of these altered the percentages to a certain extent; but as special precautions were taken and, in most cases, good-sized samples obtained, it is believed that a fair picture is presented of the nature and extent of parasitization of these primary parasites under average field conditions.

In the discussion of the primary parasites the reader is referred in each case to the paper that treats the particular species in most detail. Where no reference to literature cited is given, the information is in the form of unpublished notes on file at the Bureau of Entomology laboratory at Melrose Highlands, Mass.

Unless otherwise noted, the dead immature forms of hyperparasites that could not be positively identified, but that were encountered during the examination of the cocoons or puparia that failed to yield adults, are classed in the tables as "undetermined species."

Except for *Sturmia scutellata* R. D., all cocoons and puparia collected for this study were isolated in small glass vials stoppered with cotton plugs, which permitted accurate determinations of the percentages killed by hyperparasites.

PARASITIZATION OF PRIMARY PARASITES

HYMENOPTERA, BRACONIDAE

APANTELES LACTEICOLOR VIERECK

Apanteles lacteicolor (*5, pp. 194–201*),[3] a larval parasite of the brown-tail moth, forms its cocoons in the hibernation webs of its host about 10 days after the host larvae have begun to feed in the spring. The cocoon is very delicate and is easily crushed in handling, which may account in part for the large number of cocoons from which neither *Apanteles* nor hyperparasites were reared. Most of the cocoons of *A. lacteicolor* are formed so far in the interior of the web that to attack them the adult hyperparasites not already within it must enter and search through a labyrinth of passages for them. The extreme discomfort experienced while working with the brown-tail moth because of the irritating barbed and brittle hairs of the caterpillars has been a deterrent to the accumulation of any very large collections of its parasites. Consequently conclusions which may appear to be based on rather small numbers of cocoons or puparia are in reality founded on collections requiring a great deal of perseverance in their proper handling, and the small numbers must be considered as unavoidable under the circumstances.

In 1929 four collections totaling 457 cocoons of *Apanteles lacteicolor* were made in four localities in the New England area infested with the brown-tail moth; and in 1930 six collections totaling 1,067 cocoons were made in six localities, two in the same places as in the previous year. The percentage known to have been killed by hyperparasites of the brown-tail moth in individual collections ranged from 0 to 22, and the average for all collections in both years was 5 percent. In table 1 it is shown that 22, or 1.5 percent, of the cocoons of *A. lacteicolor* were found to have produced adults of *Eupteromalus nidulans*. Although a small percentage of the total, it represents nearly half of the number killed by hyperparasites the species of which could be determined. Yet this degree of parasitization is not surprising, for *Eupteromalus* finds these cocoons immediately available as it attains maturity following its hibernation as a primary parasite within the webs of the brown-tail moth. In fact, considering the large number of webs that were examined, and that the majority of these webs contained from a few to a hundred or more hibernating *Eupteromalus* per web, it is surprising that no greater number of *A. lacteicolor* were attacked. It is interesting that *Eupteromalus* acts both as a primary parasite of the brown-tail moth and as a secondary parasite through *A. lacteicolor* in the same web. In a previous publication (*9, p. 53*) the writer showed that *Eupteromalus* is far more important as a primary parasite under field conditions, especially when attacking the satin moth.

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[3] Reference is made by number (italic) to Literature Cited, p. 376.

TABLE 1.—*Parasitization of Apanteles lacteicolor, 1929–32*

Species of parasite	Cocoons of *A. lacteicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Eupteromalus nidulans (Thomson)	22	1.5	22	22	1.0
Monodontomerus aereus Walker	13	.9	13	13	1.0
Hemiteles tenellus (Say)	4	.3	4	4	1.0
Gelis bucculatricis (Ashmead)	2	.1	2	2	1.0
Hypopteromalus inimicus Muesebeck	2	.1	2	2	1.0
Dibrachys boucheanus (Ratzeburg)	2	.1	2	3	1.5
Eurytoma appendigaster (Swederus)	2	.1	2	2	1.0
Pleurotropis tarsalis (Ashmead) [a]	1	.1	1	1	1.0
Undetermined species	28	1.8	28		
Total killed by parasites	76	5.0			
Adults of *A. lacteicolor* issued	840	55.1			
Dead from unknown causes	608	39.9			
Total	1,524	100.0			

[a] Probably a secondary to *A. lacteicolor*

Monodontomerus aereus also spends the winter within the brown-tail-moth hibernacula, but as adults and not in such numbers as does *Eupteromalus*. Most of the individuals of *M. aereus* overwinter in the cocoon masses of the brown-tail moth, as mentioned by Muesebeck (*7, p. 447*), who adds (*p. 457*) that this species is a negligible factor in the parasitization of *A. lacteicolor*. The present figures bear out that statement.

The two most important species of secondary parasites are the ones that pass the winter in the brown-tail-moth hibernacula. This adds weight to the theory that hyperparasitism depends to a large degree on the extent of exposure.

The 5 percent mortality of *Apanteles lacteicolor* shown to be due to parasitization is certainly not all that might be charged to this, since it is at present impossible to determine with accuracy the number of primary parasites that are killed by hyperparasites when no eggs are laid or when they fail to develop. Many are killed by the hyperparasites in puncturing their primary hosts for purposes of oviposition or of feeding. For example, the writer found (*9, p. 43*) that in laboratory experiments *Eupteromalus nidulans*, acting as a primary parasite of the satin moth, laid eggs upon only one third of the total number of host larvae that it killed. Muesebeck and Dohanian (*8, pp. 6–7*) likewise show that such feeding is very common among most of the species of hyperparasites concerned in this study. It is probable that a very large number of primary parasites are killed in this way. This notation applies to all the species discussed in this paper, although generally not to such an extent as with *A. lacteicolor*, 40 percent of the cocoons of which produced neither *Apanteles* nor hyperparasites.

APANTELES MELANOSCELUS (RATZEBURG)

Muesebeck and Dohanian (*8*) studied the parasites of *Apanteles melanoscelus* (*2*), a valuable larval parasite of the gypsy moth, in considerable detail. Therefore, to avoid duplication, no special effort was made to secure a large collection of cocoons, but it was thought desirable to obtain a number sufficient for comparing the incidence of the various species of hyperparasites with their abundance when attacking other hosts during the same seasons and if possible in the same areas. In addition, the data in table 2 are presented in such a way as to show the comparative abundance of the different species when attacking each of the two generations of *A. melanoscelus*.

TABLE 2.—*Parasitization of Apanteles melanoscelus, 1929–30*

FIRST GENERATION

Species of parasite	Cocoons of *A. melanoscelus*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Eurytoma appendigaster (Swederus)	56	13.8	56	56	1.0
Hemiteles tenellus (Say)	36	8.9	36	36	1.0
Gelis bucculatricis (Ashmead)	15	3.7	15	15	1.0
Dibrachys boucheanus (Ratzeburg)	9	2.2	9	23	2.6
Mesochorus vitreus Walsh	2	.5	2	2	1.0
Gelis urbanus (Brues)	1	.2	1	1	1.0
Dimmockia incongruus (Ashmead)	1	.2	1	4	4.0
Undetermined species	12	3.0			
Total killed by parasites	132	32.5			
Adults of *A. melanoscelus* issued	210	51.7			
Dead from unknown causes	64	15.8			
Total	406	100.0			

SECOND GENERATION

Species of parasite	Cocoons of *A. melanoscelus*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Eurytoma appendigaster (Swederus)	173	69.2	[a] 156	156	1.0
Dibrachys boucheanus (Ratzeburg)	13	5.2	13	59	4.5
Gelis bucculatricis (Ashmead)	4	1.6	4	4	1.0
Eupelminus saltator (Lindeman)	1	.4	1	1	1.0
Gelis inutilis Cushman	1	.4	1	1	1.0
Habrocytus phycidis Ashmead	1	.4	1	1	1.0
Hypopteromalus inimicus Muesebeck	1	.4	1	1	1.0
Pleurotropis nawaii (Ashmead)	1	.4	1	1	1.0
Undetermined species	16	6.4			
Total killed by parasites	211	84.4			
Adults of *A. melanoscelus* issued	25	10.0			
Dead of unknown causes	14	5.6			
Total	250	100.0			

[a] Total number of cocoons giving secondary parasite adults.

In their table 1 (*8, p. 10*), Muesebeck and Dohanian showed that the first-generation cocoons of *Apanteles melanoscelus* were heavily parasitized, only 28.4 percent producing adults of *Apanteles*, whereas

52.8 percent produced hyperparasites and 18.8 percent yielded neither. These figures were based on nine collections totaling 2,164 cocoons. In the present study only 406 first-generation cocoons were collected, but they were obtained from 19 localities and during a 3-year period.

It will be seen that the total parasitization of the first generation of *Apanteles melanoscelus* here reported is 20 percent less than that found by Muesebeck and Dohanian. It is doubtful whether this difference can be attributed to periodic variation. Of course the present percentages are based on a much smaller number of cocoons collected, and thus may be less representative of the area as a whole, but on the other hand the cocoons taken by Muesebeck and Dohanian were probably obtained only in fairly heavy infestations of both the gypsy moth and its primary parasite *A. melanoscelus,* and therefore may not be as fair a sample of average conditions as is the smaller number reported here. Whatever may be the explanation, it is unlikely that it is to be found in the relative length of the period of exposure of the *Apanteles* cocoons to the attack of parasites in the two studies, for in both cases every effort was made to obtain the maximum parasitization. The methods of handling the cocoons were identical.

In the cocoons of the second generation the disparity is even greater. Again referring to Muesebeck and Dohanian's table (*8, p. 10*), the results of the first two series of second-generation collections (corresponding to the period of exposure of the present collections) were: Cocoons producing hyperparasites, 43.4 percent; cocoons producing adult *Apanteles,* 14 percent; and cocoons producing neither hyperparasites nor *Apanteles,* 42.6 percent. These percentages are based on 18 individual collections totaling 4,513 cocoons. In table 2 of the present paper the measurable amount of parasitization of *A. melanoscelus* reaches the amazing percentage of 84.4, and this is by no means the maximum, since the exposure of these cocoons was for less than half the time ordinarily experienced under field conditions. However, the percentage given by Muesebeck and Dohanian is based only on the number of cocoons producing adults of the secondaries, whereas the present percentage is obtained from cocoons which in any way were shown to have contained secondary parasites in any stage.

It is of interest to note that the two most abundant species of secondary parasites found by Muesebeck and Dohanian were likewise the most abundant in these experiments.

Parasitization in individual collections of the first-generation cocoons of *Apanteles melanoscelus* ranged from 5.3 percent in a collection of 38 to 70.3 percent in a lot of 37. In the second generation it ranged from 26.6 percent in a collection of 30 cocoons to 100 percent in a group of 149. This last collection was of interest because not a single *Apanteles* adult issued and because 143 of these cocoons produced adults of *Eurytoma appendigaster,* with the possibility that the remaining 6 cocoons which contained dead secondary parasite larvae may have been killed also by this species.

Only one tertiary parasite was reared in the work with *Apanteles melanoscelus*—a single adult of *Pleurotropis tarsalis* (Ashm.) reared from a female pupa of *Eurytoma appendigaster.* This represents only about 0.4 percent parasitization of *Eurytoma.*

APANTELES SOLITARIUS (RATZEBURG)

Among the parasites of the satin moth, one, *Apanteles solitarius*, spends the winter in two ways—as mature larvae in cocoons and as first-instar larvae in the host caterpillars. Very little information is available concerning the parasitization of the overwintering cocoons of this parasite, since they have never been plentiful. Difficulty has been experienced in obtaining summer-formed cocoons also, but the species is increasing quite rapidly, and recently several large collections have been obtained of these cocoons spun mostly by larvae which had hibernated in the host caterpillars.

HIBERNATING COCOONS

The parasitization of *Apanteles solitarius* hibernating in cocoons, as here recorded, refers only to those individuals which spent the preceding winter in the cocoons. The collections were made in the spring, and it was found that because of the weathering of the cocoons it was very difficult to distinguish those which had been formed during the previous fall from those a year older. The method adopted was to collect all cocoons not having exit holes. This probably resulted in a few old cocoons being collected and also in some of the parasitization of the generation in question being missed owing to the hyperparasites completing development and issuing before the advent of cold weather. This was hardly to be avoided, however, for in attempting to collect all the cocoons encountered and to identify the hyperparasites which had already issued, the situation would only have been made worse because of the mixing together of the cocoons formed in two different years.

A total of 44 hibernation cocoons were obtained from eight localities during the two seasons, 1930 and 1932. The results are shown in table 3. One specimen of *Pleurotropis nawaii* (Ashm.) was found dead within a pupa of one of the undetermined secondary parasites.

The parasitization in individual collections ranged from 0 to 100 percent, but the numbers of cocoons in the collections were always very small.

COCOONS OF SUMMER-ISSUING GENERATIONS

Eleven collections were made of cocoons from which the adults would issue during the current season, 1 collection in 1929, 2 in 1930, 1 in 1921, and 7 in 1932. Eight localities were included, one of which furnished a collection in each of the 4 years. Altogether, 1,526 cocoons were obtained and isolated; the results are shown in the second part of table 3. It will be noted that the percentage of cocoons producing adults of *Apanteles* is considerably higher than was the case with the hibernation cocoons, which is to be expected in view of the very much shorter period in which they were exposed to the attacks of hyperparasites. In this connection it should be stated that most of the secondary parasites reared from these cocoons were found to have developed upon pupae, whereas in the case of the hibernation cocoons, and with all the other primary parasites discussed in this paper, the development of the secondaries has been upon the larval or the nymphal stage. The writer believes that this may be explained by the fact that with these summer-issuing generations of *A. solitarius* the period spent in the cocoons is very short and the development rapid. Thus the hyperparasites on discovering the cocoons only a few days after their formation find the *Apanteles* already in the pupal stage. In the case of the most abundant secondary parasite, *Dibrachys boucheanus*, the individuals in 37, or 22.2 percent, of the cocoons were unable to complete development on the pupae thus encountered. Whether this was due to the continuation of development in the pupae after the eggs were deposited by *Dibrachys* with the consequent diminution in the available food supply for the larvae, or whether it was the result of superparasitism or of other causes is not known. About 3.7 percent of the *D. boucheanus* were killed by *Pleurotropis nawaii* acting as a tertiary in the pupae, one adult issuing from each pupa of *D. boucheanus*. An additional cocoon produced one adult of *D. boucheanus* and one of *P. nawaii*, each developing as a secondary.

TABLE 3.—*Parasitization of Apanteles solitarius, 1929 32*

IN HIBERNATION COCOONS

Species of parasite	Cocoons of *A. solitarius*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Dibrachys boucheanus (Ratzeburg)	4	9.1	4	6	1.5
Undetermined species	8	18.2			
Total killed by parasites	12	27.3			
Adults of *A. solitarius* issued	22	50.0			
Dead from unknown causes	10	22.7			
Total	41	100.0			

IN COCOONS OF SUMMER-ISSUING GENERATIONS

Species of parasite	Cocoons of *A. solitarius*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Dibrachys boucheanus (Ratzeburg)	167	10.9	[a] 130	241	1.8
Eupteromalus nidulans (Thomson)	28	1.8	28	28	1.0
Hemiteles tenellus (Say)	9	.6	9	9	1.0
Gelis bucculatricis (Ashmead)	8	.5	8	8	1.0
Gelis apantelis Cushman	7	.4	7	7	1.0
Gelis urbanus (Brues)	4	.3	4	4	1.0
Elasmus atratus Howard	3	.2	3	7	2.3
Eurytoma appendigaster (Swederus)	3	.2	3	3	1.0
Eupelminus saltator (Lindeman)	2	.1	2	2	1.0
Horismenus microgaster (Ashmead)	1	.1	1	1	1.0
Hemiteles fulvipes Gravenhorst	1	.1	1	1	1.0
Pleurotropis nawaii (Ashmead)[b]	1	.1	1	1	1.0
Undetermined species	70	4.6			
Total killed by parasites	304	19.9			
Adults of *A. solitarius* issued	974	63.8			
Dead from unknown causes	248	16.3			
Total	1,526	100.0			

[a] Total number of cocoons giving adults.
[b] Cocoon gave 1 adult of *P. nawaii* and 1 of *D. boucheanus*.

The *Apanteles* in 7 of the cocoons noted in table 3 as having died from unknown causes may have been killed by the fungus *Beauveria ? globulifera* (Speg.) Pic.[4] This is known to be a species capable of acting either as a parasite or as a saprophyte, and although it is considered by some mycologists to be primarily parasitic, there is no evidence in this case to indicate its true status.

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[4] Identification and information by M. T. Smulyan, formerly of the Bureau of Entomology, laboratory, Melrose Highlands, Mass.

In table 3 *Eupteromalus nidulans* is shown as attacking *Apanteles solitarius* to the extent of 1.8 percent. This is probably due to the fact that *Eupteromalus*, in hibernating in the satin-moth webs, finds these cocoons available very soon after its issuance in the spring. It may pass one or more generations while acting as a parasite of *A. solitarus* before it again turns to its more important role as a primary parasite of the satin moth. In the period covered by these studies, its importance as a parasite of *A. solitarus* is indicated by its attack of 1.8 percent of the summer-issuing cocoons, while its value as a primary parasite of the hibernating satin-moth larvae is shown by its parasitization of 28 percent in all webs examined.

The hyperparasitism in individual collections ranged from 0 in a lot of 18 cocoons to 30.8 percent in a collection of 13.

METEORUS VERSICOLOR (WESMAEL) AS A BROWN-TAIL MOTH PARASITE

As a parasite of the brown-tail moth *Meteorus versicolor* (*5, pp. 201-205*) spends the winter as a first-instar larva within the partly grown caterpillar of its host. Development is completed in the spring, and the cocoons of *Meteorus* are each suspended by a long thread from the branches of the tree on which the host larvae were feeding. The cocoons are so easily moved about by the wind and the foothold is so unstable that it is strange that the hyperparasites are able to complete oviposition successfully. The process was observed several times in the case of *Hemiteles tenellus*, where, with much beating of wings and repeated short flights when the balance was upset, it was finally completed.

Although *Meteorus* has more than one generation upon the brown-tail moth as host each year, it seems desirable to treat the cocoons of the various generations as a unit, owing to the tendency of the generations to overlap and the consequent difficulty of distinguishing between them, together with the general scarcity of the cocoons in the field and the resulting rather small total collected. As shown in table 4, 357 cocoons were examined. These were obtained in 15 collections from 7 localities over a period of 4 years.

TABLE 4.—*Parasitization of Meteorus versicolor, 1929–32*

Species of parasite	Cocoons of *M versicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Hemiteles tenellus (Say)	60	16.8	60	60	1.0
Eurytoma appendigaster (Swederus)	28	7.8	28	28	1.0
Cirrospilus cinctithorax (Girault)	3	.8	3	27	9.0
Monodontomerus aereus Walker	2	.6	2	3	1.5
Eupteromalus nidulans (Thomson)	2	.6	2	2	1.0
Dibrachys boucheanus (Ratzeburg)	1	.3	1	1	1.0
Eupelmus spongipartus Foerster	1	.3	1	1	1.0
Gelis urbanus (Brues)	1	.3	1	1	1.0
Habrocytus phycidis Ashmead	1	.3	1	1	1.0
Thysiotorus triangularis (Cresson)	1	.3	1	1	1.0
Undetermined species	8	2.2			
Total killed by parasites	108	30.3			
Adults of *M. versicolor* issued	215	60.2			
Dead from unknown causes	34	9.5			
Total	357	100.0			

In table 5 are given the results of some earlier collections made by various members of the laboratory staff and reared by C. F. W. Muesebeck and S. M. Dohanian. In comparing the figures in tables 4 and 5 it is to be noted that the evident parasitization was greater in the cocoons collected for the present study, but this is not strictly true, for the degree of parasitization expressed in table 5 probably does not represent the whole amount but only that of the cocoons from which adult hyperparasites issued. As a consequence, part of the larger percentage of individuals included in the grouping "Dead from unknown causes" (table 5) may have been killed by secondary parasites. The difference between the percentages of cocoons yielding adults of *Meteorus* in tables 4 and 5 may or may not be significant.

TABLE 5.—*Parasitization of Meteorus versicolor, 1921–22* [a]

Species of parasite	Cocoons of *M. versicolor*		Issuance of parasites		
			Host cocoons concerned	Total adults issued	Average adults per cocoon
	Number	*Percent*	*Number*	*Number*	*Number*
Hemiteles tenellus (Say)	94	14.7	94	94	1.0
Dibrachys boucheanus (Ratzeburg)	[b] 27	4.2	1	3	3.0
Monodontomerus aereus Walker	6	.9	6	8	1.3
Eupelminus sp [c]	5	.8	5	5	1.0
Gelis sp [c]	2	.3	2	2	1.0
Habrocytus sp [c]	1	.2	1	1	1.0
Hemiteles sp [c]	1	.2	1	1	1.0
Mesochorus sp [c]	1	.2	1	1	1.0
Undetermined species [c]	12	1.8	4	31	7.8
Total killed by parasites	149	23.3			
Adults of *M. versicolor* issued	341	53.3			
Dead from unknown causes	[d] 150	23.4			
Total	640	100.0			

[a] These collections were made by various members of the laboratory staff. The rearing and identification of the secondary parasites were by C. F. W. Muesebeck and S. M. Dohanian.

[b] Total number of cocoons for which the records are complete as regards the number of secondary parasite adults to issue from each.

[c] The specimens cannot now be located for specific identification.

[d] It is doubtful whether the cocoons from which nothing issued were examined for the presence of dead immature forms. Thus some parasitization is probably included in the category "Dead from unknown causes."

A further comparison of these tables shows that *Hemitelès tenellus* is by far the most abundant secondary parasite in both sets of data, but that the remaining species common to both tables are variable in their relative abundance.

The cocoons which furnished the data for table 5 were obtained in 14 collections from 8 localities during 2 seasons. The parasitization in individual collections ranged from 4.5 percent in a lot of 88 cocoons to 59.2 percent in a lot of 71.

In the present study the parasitization in individual collections ranged from 0 in very small collections to 95.2 percent in a lot of 21, where the *Meteorus* in 20 of the cocoons were killed by secondaries and the remaining cocoon may have been so, as no *Meteorus* issued.

AS A SATIN-MOTH PARASITE

Since a species of *Meteorus* known to be morphologically similar to *M. versicolor* was found by United States Department of Agriculture workers at the Bureau of Entomology laboratory at Budapest, Hungary, to be a valuable parasite of the satin moth at various points in Europe, many cocoons of this species were collected and sent to this country, where the adults were liberated. Subsequently individuals of what are believed to be this species have been recovered in increasing numbers from both hibernating and summer-feeding satin-moth larvae in New England. C. F. W. Muesebeck, after a careful study of the adults from both the brown-tail moth and the satin moth as hosts, stated[5] that in his opinion they are indistinguishable, although certain details in the biology of those from the satin moth (such as the proportion of sexes) apparently differ from those of the individuals from the brown-tail moth.

Only 9 cocoons have been found and isolated, and of these 6 produced adults of *Meteorus*, 1 an adult of *Hemiteles tenellus*, 1 an adult of *Gelis apantelis;* the *Meteorus* in the remaining cocoon died from an unknown cause. Both hyperparasites were reared from a collection of 5 cocoons made in 1932. It is probable that the degree and nature of the parasitization of *Meteorus versicolor* when it is a parasite of the satin moth will be found to be much the same as when it is a parasite of the brown-tail moth.

HYMENOPTERA, PTEROMALIDAE

EUPTEROMALUS NIDULANS (THOMSON)

As pointed out in a previous paper (*9*), *Eupteromalus nidulans* is of much greater importance as a primary parasite, though capable of acting either as a primary or a secondary. This importance has been more evident in the last few years in its actions as a primary upon both the satin moth (its chief host) and the brown-tail moth. In table 6 it can be seen that the numbers of hibernating *Eupteromalus* found in brown-tail-moth hibernacula have greatly increased since 1928, although the number of hibernacula examined each year has remained about the same (about 250, not taking into account the large collections made for colonization purposes as indicated in a footnote to the table).

TABLE 6.—*Parasitization of Eupteromalus nidulans*

Date of collection, winter of—	E. nidulans collected—		Parasitization by—			
	In webs of brown-tail moth	In webs of satin moth	*Pleurotropis nawaii*		*Pleurotropis tarsalis*	
			Number	*Percent*	*Number*	*Percent*
1926–27	[a] 90					
1927–28	[a] 84		2	2.4		
1928–29	[a] 544					
1929–30	709					
1930–31	959		201	21.0		
1931–32	795		27	3.4		
1930–31 [b]	1,769		441	[c] 24.9		
1931–32 [b]	3,785		982	[c] 25.9	3	0.1
1931–32		56	6	10.7		
Total	8,735	56	1,659	18.9	3	

[a] The notes on some of these earlier collections are incomplete in regard to the amount of parasitization and to the species concerned. The figures given here represent only the opinion of the writer after examining the available data.

[b] The issuance from these collections of the adults of *E. nidulans* and of its parasites was delayed by refrigeration until the middle of the summer of 1931 and 1932, respectively, so that newly issued adults of *E. nidulans* would be produced at the desired time for colonization purposes.

[c] Based on total number of issuing adults of *Eupteromalus* and *Pleurotropis*.

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[5] In a letter to the writer, dated Nov. 15, 1932.

In table 6 it will be noted that *E. nidulans* became abundant in the webs collected during the winter of 1928–29, but that the hyperparasite *Pleurotropis nawaii* did not appear in large numbers in the collections until 2 years later, although from its abundance at that time it seems likely that it had been increasing previously and simply had not been obtained in the samples taken. *P. tarsalis* apparently occurs only rarely as a parasite of *Eupteromalus*. The percentage of parasitization by both species of *Pleurotropis* based on the total number of *Eupteromalus* collected in the entire period is 18.9. This percentage is somewhat smaller than those obtained from the large collections of brown-tail-moth webs made for colonization purposes.

Comparatively little is known of the extent of parasitization of *Eupteromalus* when it is a primary parasite of the satin moth. General observations indicate that under these conditions it has always been rather negligible. During the winter of 1931–32 *Pleurotropis nawaii* was found attacking *Eupteromalus* at 3 of the 11 localities where the latter was parasitic upon the satin moth, when the percentage of parasitization was only 10.7 for the area as a whole. At these three points *Eupteromalus* had been abundant in the hibernacula of this host during the previous 2 or 3 years.

DIPTERA, TACHINIDAE

COMPSILURA CONCINNATA MEIGEN

The tachinid *Compsilura concinnata* (*3*) is one of the most important parasites of the gypsy moth, the brown-tail moth, and the satin moth, and in addition it attacks a variety of other hosts of lesser economic importance. Although the puparia of this species are usually formed at or just below the surface of the ground, as with most tachinids, yet when *C. concinnata* is a parasite of the brown-tail moth, the puparia are normally formed near the host itself, the larvae not dropping to the ground. This is true to a lesser extent when the gypsy moth is the host. As a result, a great many more puparia were obtained from the cocoon masses of the brown-tail moth than from any other host.

AS A BROWN-TAIL MOTH PARASITE

A total of 771 puparia of *Compsilura* as a parasite of the brown-tail moth was obtained in 39 collections from 18 localities over a period of 4 years. As indicated by these figures, many of the collections were small, but even among the larger lots the parasitization ranged from 0 in a collection of 28 puparia to practically 100 percent in a lot of 32, where 31 were found to have been killed by *Monodontomerus aereus* and the other died from an unknown cause. The average percentage of parasitization is very high, as shown in table 7, *M. aereus* causing more than two thirds of the total amount. This species has long been known to be an important parasite of *Compsilura*, but to what extent had never been determined until the present study was made. Its increase is made the greater by its ability to mature an average of 7.8 adults to each puparium of its host. Of the 2,047 adults reared, 1,060 were males and 987 females.

Monodontomerus aereus in turn was parasitized by *Pleurotropis nawaii*, 89 individuals, or 4.2 percent of the total, having been reared as against 2,047 adults of *Monodontomerus*.

TABLE 7.—*Parasitization of Compsilura concinnata when it is a parasite of the brown-tail moth, the gypsy moth, and the satin moth, 1929–32*

AS A PARASITE OF THE BROWN-TAIL MOTH

Species of parasite	Puparia of *Compsilura*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number* [a]	*Number*	*Number*
Monodontomerus aereus Walker	324	42.0	204	2,047	7.8
Eurytoma appendigaster (Swederus)	27	3.6	24	24	1.0
Dibrachys boucheanus (Ratzeburg)	14	1.8	12	192	16.0
Brachymeria compsilurae (Crawford)	11	1.4	11	11	1.0
Psychophagus omnivorus (Walker)	5	.6	4	25	6.2
Undetermined species	80	10.4			
Total killed by parasites	461	59.8			
Adults of *Compsilura* issued	196	25.4			
Dead from unknown causes	114	14.8			
Total	771	100.0			

AS A PARASITE OF THE GYPSY MOTH

Species of parasite	Puparia of *Compsilura*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number* [a]	*Number*	*Number*
Dicrachys boucheanus (Ratzeburg)	21	6.8	21	214	10.2
Brachymeria compsilurae (Crawford)	17	5.6	17	17	1.0
Miotropis clisiocampae Ashmead	1	.3			
Undetermined species	11	3.6			
Total killed by parasites	50	16.3			
Adults of *Compsilura* issued	182	59.5			
Dead from unknown causes	74	24.2			
Total	306	100.0			

AS A PARASITE OF THE SATIN MOTH

Species of parasite	Puparia of *Compsilura*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number* [a]	*Number*	*Number*
Brachymeria compsilurae (Crawford)	1	11.1	1	1	1.0
Phygadeuon subfuscus Cresson	1	11.1	1	1	1.0
Dibrachys boucheanus (Ratzeburg)	1	11.1	1	1	1.0
Psychophagus omnivorus (Walker)				1	1.0
Undetermined species	1	11.2			
Total killed by parasites	4	44.5			
Adults of *Compsilura* issued	3	33.3			
Dead from unknown causes	2	22.2			
Total	9	100.0			

[a] Total number of puparia giving secondary parasite adults

Dibrachys boucheanus was also attacked by *Pleurotropis nawaii*, 192 adults of *Dibrachys* having been reared and 13 individuals of *Pleurotropis*, a parasitization of 6.3 percent.

One adult of *Pleurotropis tarsalis* was reared as a parasite of *Eurytoma appendigaster*, which would represent a parasitization of 4 percent.

AS A GYPSY-MOTH PARASITE

Considerably less is known regarding the parasitization of *Compsilura* when its host is the gypsy moth. The puparia are very difficult to find unless both host and parasite are especially abundant; and even then, in several cases where special efforts were made, none could be found in or on the ground. A total of 306 puparia have been obtained in five collections from as many localities over a period of 3 years. In the second part of table 7 the percentage of parasitization of *Compsilura* as a gypsy-moth parasite is shown to be considerably lower than was the case when *Compsilura* was parasitic upon the brown-tail moth. While based on a much smaller number of puparia, this percentage is not greatly different from the parasitization of *Compsilura* when a parasite of the brown-tail moth if that part due to *Monodontomerus* is subtracted. In this case the primary lepidopterous host is one not especially subject to the attack of *Monodontomerus*. In fact *Monodontomerus* was not reared at all from these collections, although it is very general in its hyperparasitic habits.

The parasitization in individual collections varied greatly, but that of one large collection totaled 15.5 percent.

A record of the rearing of *Conostigmus* n.sp. from a puparium of *Compsilura* is on file at the Melrose Highlands laboratory, the puparium probably having been obtained from the gypsy moth as host.

AS A SATIN-MOTH PARASITE

As a parasite of the satin moth, *Compsilura* has been abundant at several points in the infested area in New England, but despite considerable effort practically not puparia could be collected at these points. As a result only 9 puparia were obtained in 2 collections from 2 localities, made in different years; the first collection consisting of puparia from the surface of the ground and the second of puparia taken from "spin-ups" (the slight webbing spun by the host larva to hold it to the leaf during the pupal period). The third part of table 7 shows the parasitization in these 9 puparia, and while in no way conclusive, it does indicate that about the same species of hyperparasites, except for *Monodontomerus*, attack *Compsilura* when it is a parasite of the satin moth as when it parasitizes the gypsy moth or the brown-tail moth.

One interesting point is the rearing of an adult of *Dibrachys boucheanus* and one of *Psychophagus omnivorus* from the same puparium, where apparently neither interfered with the development of the other, both species being generally gregarious.

EXPERIMENTAL EFFORTS

Because so few puparia were obtained at infestations of the gypsy moth and the satin moth, newly formed puparia of *Compsilura concinnata* were taken from the laboratory into the field at infestations of these hosts and exposed to the attack of hyperparasites. A total of 680 puparia were exposed in various ways at several infestations. After a period of 1 week careful search resulted in the finding of only 113 puparia intact, the others having been destroyed by an unknown agency. No hyperparasites were reared from any of these 113 puparia.

STURMIA NIDICOLA (TOWNSEND)

The important parasite *Sturmia nidicola* (*6*) is specific upon the brown-tail moth, forming its puparium within the larval skin of the prepupal host larva. It spends the winter as a first-instar larva within the esophagus of its host, from which it migrates when feeding is resumed in the spring. A total of 1,331 puparia were obtained in 31 collections from 17 localities in the New England area infested with its host, from 1929 to 1931, inclusive. Table 8 gives the results obtained from these collections.

TABLE 8.—*Parasitization of Sturmia nidicola, 1929-31*

Species of parasite	Puparia of *S. nidicola*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number*	*Number*	*Number*
Monodontomerus aereus Walker	129	9.7	[a] 122	739	6.0
Brachymeria compsilurae (Crawford)	62	4.7	62	62	1.0
Itoplectis conquisitor (Say)	1	.1	1	1	1.0
Undetermined species	122	9.1			
Total killed by secondary parasites	314	23.6			
Adults of *S. nidicola* issued	527	39.6			
Dead from unknown causes	490	36.8			
Total	1,331	100.0			

[a] Total number of puparia producing secondary parasite adults

It will be noted that 36.8 percent of all puparia collected were "dead from unknown causes." This high unexplained mortality was evident in all collections and amounted to over half in some. It is not known whether this was produced by the oviposition activities of *Monodontomerus aereus* and other secondaries, but it is suspected that such was the case, at least in part.

Monodontomerus aereus is again the most abundant hyperparasite.

STURMIA SCUTELLATA (ROBINEAU DESVOIDY) [6]

Sturmia scutellata, a large tachinid, is practically a specific parasite of the gypsy moth in New England, laying its eggs on the foliage, where they are eaten by the larvae of about the fourth instar. The maggots, when they have completed development, issue from the pupae of the host and drop to the ground, which they enter to a depth of 2 or 3 inches. They pupate and remain in the ground until the following May or June, when the adults emerge. Parasitization therefore takes place either before the *Sturmia* larvae leave the host or after they have burrowed into the earth. It is known positively that in the case of two species mentioned in table 9 (*Brachymeria* and *Perilampus*) the attack occurs before the maggots of *S. scutellata* have left the host pupae. Such may be the case with *Conostigmus* and *Phygadeuon* also, although this point could not be proved, as the puparia in the collections from which these species issued were allowed to remain in the ground for some time after the maggots had entered or else were kept in containers which hyperparasites might have entered, and hence the attack could have occurred after the puparia were formed.

One thousand three hundred puparia were obtained in 10 collections from 6 localities over a period of 5 years, as shown in table 9. Most of these puparia were obtained by making collections of gypsy-moth pupae from localities in which *Sturmia scutellata* was known to be abundant. These pupae were concentrated around the base of a tree over a piece of wire screening sunk about 1 foot below the surface of the ground. The earth was later sifted for the puparia which had been formed by the larvae thus allowed to leave the hosts and enter the soil in a nearly natural manner. The puparia were then carried through the period of hibernation in soil or in jars with provision for an adequate moisture supply.

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[6] Practically all the work with this species was done by T. H. Jones and assistants, of the Bureau of Entomology, Melrose Highlands, Mass.

TABLE 9.—*Parasitization of Sturmia scutellata, 1927–31*

Species of parasite	Puparia of *S. scutellata*		Issuance of parasites		
			Host puparia concerned	Total adults issued	Average adults per puparium
	Number	*Percent*	*Number*	*Number*	*Number*
Brachymeria compsilurae (Crawford)	121	9.3	[a] 117	117	1.0
(*Megaspilus*) *Conostigmus virginicus* (Ashmead)	24	1.8	24	224	9.3
Perilampus hyalinus Say [b]	1	.1	1	1	1.0
Phygadeuon subfuscus Cresson	1	.1	1	1	1.0
Total killed by parasites	147	11.3			
Adults of *S. scutellata* issued	500	38.5			
Dead from unknown causes	653	50.2			
Total	1,300	100.0			

[a] Total number of puparia giving secondary parasite adults

[b] Determined by P. B. Dowden, of the Bureau of Entomology laboratory, Melrose Highlands, Mass.

It is seen in table 9 that *Sturmia scutellata* is not extensively parasitized. Probably the figures given do not represent the full amount of parasitization, as no puparia failing to yield adults of *S. scutellata* or its parasites were examined for the cause of death except in 1932. The numbers omitted would add only slightly to the total number killed by parasites, however, since the greater part of those recorded as "dead from unknown causes" may well have been those individuals which failed to hibernate successfully, as it is known that there is considerable mortality from this cause. *Brachymeria compsilurae* is by far the most important parasite of *Sturmia scutellata*.

In table 10 are brought together all the available data concerning the abundance of *Brachymeria* obtained in experiments performed by various members of the laboratory staff. These experiments were carried out for other purposes, and no attention was paid to any hyperparasites other than *B. compsilurae*. The huge total of 24,070 puparia was obtained over a period of 4 years and from many localities, so the percentage yielding *Brachymeria* is very dependable. This is not greatly different from the percentage shown in table 9.

TABLE 10.—*Parasitization of Sturmia scutellata by Brachymeria compsilurae, 1925–27 and 1931*

Item	Puparia of *S. scutellata*	
	Number	*Percent*
Killed by *Brachymeria compsilurae*	1,938	8.1
Adult *S. scutellata* issued	11,586	48.1
Dead from unknown causes	10,546	43.8
Total	24,070	100.0

CARCELIA LAXIFRONS VILLENEUVE

The tachinid *Carcelia laxifrons* (*1, p. 136*) has never been abundant enough to permit the making of large collections, but its puparia are occasionally encountered in the pupal "spin-ups" of the brown-tail moth. Eighteen puparia were obtained in 4 collections from as many points from 1929 to 1931, inclusive. Eight puparia produced adult *Carcelia*, 2 yielded hyperparasites, and the remaining 8 pupae died from unknown causes. Of the 2 killed by secondaries, 1 produced *Brachymeria compsilurae* and the other was found to contain many dead larvae of *Monodontomerus aereus.*

It is probable that the puparia of *Carcelia laxifrons* will be found to be attacked by the same species of parasites and about as often as are those of *Compsilura* when the latter is acting as a parasite of the brown-tail moth.

TACHINA MELLA WALKER

The only native primary parasite discussed in the present paper is *Tachina mella* (*1, p. 112*), the others all having been imported for the control of the gypsy moth, brown-tail moth, satin moth, or oriental moth. Only three puparia of this species have been collected, and in each case it was a parasite of the brown-tail moth; these were found in different years. *T. mella* occurs but rarely as a parasite of the brown-tail moth or at least the puparia are not readily found. Of the 3 puparia collected, 1 produced an adult fly, 1 yielded 6 adults of *Monodontomerus aereus*, and the third pupa died from an unknown cause.

CHAETEXORISTA JAVANA BRAUER AND BERGENSTAMM

An important tachinid parasite of the oriental moth has been liberated only recently, but has gained a foothold so quickly that it was possible to obtain 61 puparia in three collections from the infested area of New England in 1932. This tachinid is *Chaetexorista javana.* Every puparium yielded an adult fly.

The puparium of the parasite is formed in the spring within the hibernation cocoon of its host, the latter being so very hard and thick that it is broken only with considerable difficulty. Under these conditions it is not surprising that no hyperparasites were reared. However, 1 of 78 cocoons of the oriental moth collected for another purpose at Jamaica Plain, Mass., in 1930, was found to contain a puparium of *Chaetexorista* which, when examined some time later, contained many dead adults of a species of *Melittobia.* Possibly the attack occurred in the laboratory, as a member of this genus (*M. acasta* Walker) is known to be a laboratory pest (*4, p. 209*), but apparently the present species is able to penetrate the cocoon and puparium to effect oviposition. Perhaps other species may be found to do the same in spite of the nature of the cocoon.

CONCLUSIONS

From these investigations it may be deduced that hyperparasites of the gypsy moth, the brown-tail moth, the oriental moth, and the satin moth play a considerable part in the reduction of the numbers of the primary parasites here considered, a general average of almost one third of the cocoons and puparia being destroyed by them.

In almost every case, each primary parasite has one species of secondary parasite which is far more abundant than any other. In general, this was found to be true in the individual collections and from year to year. Except in the case of the tachinids, where one might expect the stimulus for oviposition in the puparia of the different species to be much the same, the chief hyperparasitic species was not the same for any two primary parasites.

A summary of the 10 preceding tables is given in table 11 with the inclusion of the corresponding data on *Carcelia laxifrons* and *Tachina mella.*

TABLE 11.—*Summary of parasitization in New England of the primary parasites of the gypsy moth, the brown-tail moth, the satin moth, and the oriental moth*

Primary parasite	Adult primaries issued		Primaries attacked by secondaries		Dead from unknown causes	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Apanteles lacteicolor	840	55.1	76	5.0	608	39.9
Apanteles melanoscelus I	210	51.7	132	32.5	64	15.8
Apanteles melanoscelus II	25	10.0	211	84.4	14	5.6
Apanteles solitarius, winter cocoons	22	50.0	12	27.3	10	22.7
Apanteles solitarius, summer generations	974	63.8	304	19.9	248	16.3
Meteorus versicolor, 1921–22	341	53.3	149	23.3	150	23.4
Meteorus versicolor, 1929–32	215	60.2	108	30.3	34	9.5
Eupteromalus nidulans	-----	-----	1,662	18.9	-----	-----
Compsilura concinnata on brown-tail moth	196	25.4	461	59.8	114	14.8
Compsilura concinnata on gypsy moth	182	59.5	50	16.3	74	24.2
Compsilura concinnata on satin moth	3	33.3	4	44.5	2	22.2
Sturmia nidicola	527	39.6	314	23.6	490	36.8
Sturmia scutellata, 1927–31	500	38.5	147	11.3	653	50.2
Sturmia scutellata, various records	11,586	48.1	1,938	8.1	10,546	43.8
Carcelia laxifrons	8	44.4	2	11.1	8	44.5
Tachina mella	1	33.3	1	33.3	1	33.4

SUMMARY

Only 5 percent of the cocoons of *Apanteles lacteicolor* were proved to have been killed by secondary parasites, although not all the parasitization was measurable. Adult *Apanteles* issued from 55.1 percent of the total number collected, which is a higher percentage than is found in many of the other parasites. *Eupteromalus nidulans* was the chief hyperparasite.

Parasitization of the cocoons of *Apanteles melanoscelus* was found to be 32.5 percent in the first generation and 84.4 percent in the second. No effort was made to obtain a large collection of the second-generation cocoons, but of the small number received, adult *A. melanoscelus* issued from 10 percent. *Eurytoma appendigaster* was the most abundant parasite of the cocoons of both generations.

Hibernation cocoons of the parasite *Apanteles solitarius* yielded 27 percent of hyperparasites, and those formed during the summer showed 20 percent attack by secondaries. Adults of *A. solitarius* issued from half of the hibernation cocoons and from 64 percent of those of the summer generations. *Dibrachys boucheanus* was by far the most abundant parasite of the cocoons of all generations.

Thirty percent of the cocoons of *Meteorus versicolor* were killed by parasites, and 60 percent yielded adults of *Meteorus*. *Hemiteles tenellus* was the chief parasite.

Of the individuals of *Eupteromalus nidulans* collected from all sources, 19 percent were killed by secondaries, and most of the remainder issued as adults. *Pleurotropis nawaii* was the only secondary parasite of any importance.

The parasitization of *Compsilura concinnata* is presented as found in relation to the three important primary hosts. As a parasite of the brown-tail moth, *C. concinnata* was attacked chiefly by *Monodontomerus aereus;* as a parasite of the gypsy moth, chiefly by *Dibrachys boucheanus;* and as a parasite of the satin moth, in the few cases found, by several equally.

Parasites killed 23.6 percent of the puparia of *Sturmia nidicola*, and adult *Sturmia* issued from 40 percent. *Monodontomerus aereus* was the most common secondary parasite.

The parasitization of *Sturmia scutellata* was found to be 11 percent, with *Brachymeria compsilurae* responsible for 9 percent of it in the present study and 8 percent when based on a total number of 24,070 puparia obtained in another experiment. Adult *S. scutellata* issued from 38.5 percent and 48 percent of the puparia in the two groups.

Eighteen puparia of *Carcelia laxifrons* collected showed 2 (11 percent) parasitized, 1 each by *Brachymeria compsilurae* and *Monodontomerus aereus*. Adult *Carcelia* emerged from 8 of the remaining.

Three puparia of *Tachina mella* yielded 1 adult fly, 1 killed by *Monodontomerus aereus*, and 1 dead from an unknown cause.

No parasitization was found in the puparia of *Chaetexorista javana*, although a single puparium obtained in another experiment contained adults of a species of *Melittobia*.

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THE RELATION OF "DARK CENTER" TO THE COMPOSITION OF RUTABAGAS[1]

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INTRODUCTION

"Dark center" of rutabaga (*Brassica campestris* L.) is a well-known disorder that occurs in New England, Canada, and portions of Europe and New Zealand. It has become so prevalent in certain sections of Massachusetts as to destroy the marketability of a large percentage of the rutabaga crop.

Dark center is characterized by irregular dark streaks in the parenchyma of the rutabaga, with the formation of more or less stringy fibers, and in some instances a water-soaked area; hence the names "mottled heart", "water core", etc. These changes develop rapidly about the time of harvesting, but have not been correlated with any fungous or bacterial disease so far as noted. W. H. Davis, of Massachusetts State College, made cultures on agar and found the tissue sterile. He did find, however, by microscopical examination, a break-down of the cell walls similar to edema, which would seem to indicate a functional disturbance due to environmental conditions.

ANALYSES

Bushel samples of supposedly normal and affected Purple Top rutabagas were drawn from a field in North Eastham, Mass., in 1932 and submitted to the laboratory for analysis. The roots averaged 922 g in weight. Of the 28 apparently normal roots, 16 showed dark streaks when halved vertically, and these were excluded. The 31 affected rutabagas contained 1 marketable root, which was likewise excluded. Neither fibrous formations nor water-soaked areas were observed in the 30 specimens retained. These are probably symptoms of a more advanced stage of the disorder.

The selected rutabagas were sliced, dried quickly, ground to pass a 1-mm sieve, and produced satisfactory yellowish meals of similar appearance except that the meal from the affected roots was slightly darker. The two samples were analyzed by the ordinary methods. The results are shown in table 1.

As compared with averages published in various compilations, the normal rutabagas were high in carbohydrates (nitrogen-free extract), especially total sugars, and correspondingly low in protein, fiber, and ash. They were of excellent quality as a table product. The roots affected by dark center suffered a substantial loss in nitrogen-free extract, with an increase in protein, fiber, and ash. The loss in total sugars was partly compensated by gains in pentosans and galactan (gums).

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[2] The writers are indebted to G. B. Snyder and R. W. Donaldson, of the Massachusetts State College, for data relative to the dark-center disorder and its distribution.

TABLE 1.—*Analyses (percent) of Purple Top rutabagas when normal and when affected with dark center*

[Results on dry-matter basis]

Constituent	Normal	Affected with dark center	Constituent	Normal	Affected with dark center
Original moisture	89.70	88.72	Nitrogen-free extract—Continued.		
Crude protein (N×6.25)	5.94	9.21	Pentosans	7.39	9.89
Total nitrogen	.95	1.47	Galactan	2.79	6.03
Amino nitrogen (Van Slyke)	.11	.18	Crude fiber (cellulose)	7.28	10.85
Nitrate nitrogen	([a])	([a])	Crude ash	4.07	5.63
Crude fat (ether extract)	1.31	1.20	Acid-soluble ash	4.04	5.63
Nitrogen-free extract	81.40	73.11	Acid-insoluble ash	.03	00
Dextrose	48.53	36.99	pH at 25° C	5.75	5.63
Sucrose	12.17	10.87			
Residual carbohydrates as starch [b]	7.47	7.90			

[a] None.

[b] Little, if any, true starch present.

The initial effect of dark center is the dissipation of an appreciable proportion of the sugars, but to what purpose it is at present impossible to state.

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SIZE, SHAPE, AND ORIENTATION OF PLOTS AND NUMBER OF REPLICATIONS REQUIRED IN SWEETPOTATO FIELD-PLOT EXPERIMENTS[1]

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INTRODUCTION

The ultimate object of field-plot experiments is not only to record the differences in behavior of plants of different kinds or of plants subjected to various treatments, but also to determine whether those differences in behavior are really significant, whether they are due to the treatments applied, or whether they are the result of some chance disturbing factor. To do this it is necessary to employ various methods for estimating the error that is certain to attend experiments of this kind. The many sources of variation that may contribute to error in results from such experiments make it essential that every available means be utilized to eliminate or reduce error in plot yields.

Numerous investigations conducted on field-plot technic during the last decade have demonstrated the importance of size, shape, and replication of plots in reducing error. The value of statistical methods in interpreting results from field experiments has become widely recognized. Those variations due to chance can be satisfactorily accounted for by statistical methods, but it must be borne in mind that errors due to poor technic cannot be removed by statistical analyses.

Salmon (*8*)[3] has pointed out some of the limitations of statistical interpretation of results from field experiments and has emphasized the importance of good judgment on the part of the experimenter. Judgment and care in selecting uniform land, in handling field operations, and in the technic used in measuring, weighing, and computing yields must be exercised, or statistical treatment of the results will be of no avail. The application of statistics to yield records will not eliminate errors resulting from careless methods in planting and in harvesting and recording yields. It is only when careful attention has been given to these operations that statistical treatment of the results can be expected to give trustworthy information. Under any other condition statistical interpretations may be misleading.

Before any extensive field experiments with a crop are made, it is important that the size of plot and the number of replications required to give accurate results be ascertained. In the spring of 1929 the experimental work outlined in this paper was begun for the purpose of determining the size and shape of plot and the number of replications

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[2] The writer is indebted to G. N. Collins, Division of Genetics and Biophysics, and C. S. Scofield, Division of Western Irrigation Agriculture, for reading and criticizing the manuscript, and to A. M. Jackson, formerly scientific aid in the Division of Fruit and Vegetable Crops and Diseases, for assistance in the mathematical computations.

[3] Reference is made by number (italic) to Literature Cited, p. 399.

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necessary to reduce the error in plot experiments with sweetpotatoes to the lowest limit possible under field conditions.

REVIEW OF LITERATURE

A survey of the literature on the standardization of field experiments shows this to have been a fertile field for investigation during the last 20 years, as indicated by the number of articles published in scientific journals. No attempt will be made here to review the entire field; only a few articles having a direct bearing on this problem will be mentioned.

Wood and Stratton (*10*) in 1910 were among the first to apply statistical methods in interpreting results from field-plot experiments. They emphasized the need for caution in interpreting results from field experiments and outlined methods for statistical analyses. They determined the probable error of results from field plots of mangels by two methods and found it to be about 5 percent of the crop. The experimental error they found to be independent of size of plot, provided a plot of one eightieth of an acre or larger was used. By statistical analysis they determined the number of plot replications necessary to give significant reduction in error.

Mercer and Hall (*6*) in 1911 applied statistical methods to field experiments with mangels and wheat in determining the size of plot and the number of replications necessary to reduce the probable error to practicable limits. They found that the error diminished with the increase in size of the plot, but the difference in error was of little significance when the plot size was increased above one fortieth of an acre. By increasing the number of similarly treated plots and scattering them over the experimental area, they found the reduction in the probable error to be significant up to five replications, beyond which the change was insignificant. For practical purposes in field experiments they recommended the use of five plots of one fortieth of an acre each, systematically distributed over the area to be used for the experiment. They concluded that there is little difference in error between long narrow plots and square ones.

Day (*3*) in 1920, in a study of three thousand one hundred 5-foot row units of Fulcaster wheat, concluded that where plots of a given area are to be used the greatest accuracy can be obtained from long narrow plots lying in the direction of the greatest soil variation. Where the experimental area showed about equal variation in length and width, the shape of plot was found to have no influence on the results. Units of comparison composed of systematically distributed plots gave much less variable results than an equal area in a single plot.

Westover (*9*) in 1924 determined the size of plot and the number of replications necessary in field experiments with potatoes under conditions such as exist in the potato-growing districts of West Virginia. He concluded that reliable results may be obtained from the use of four systematically distributed 40-foot single-row plots.

Christidis (*2*) in 1931, in a general discussion of the question of the effect of shape of plot on the reduction of error in field experiments, presented data from various sources and made statistical comparisons between field plots of various shapes. In no instance did he find square plots to be significantly more uniform than long narrow ones.

In cases where the long narrow plots did not show an advantage over square plots, there was no indication in favor of either shape. The advantage of long narrow plots over square plots was found to be less significant when the greater dimension of the plots ran at right angles to the direction of the rows. He considered a very small factor for the ratio of width to length of plot to be desirable for yield trials from a theoretical standpoint. However, he concluded that there are some practical considerations which may make it desirable to increase the width of plot in relation to its length.

MATERIAL AND METHODS

The yield records used in the present studies were taken from four plantings of sweetpotatoes (*Ipomoea batatas* (L.) Lam.) of the variety Porto Rico, grown during the seasons of 1929, 1930, and 1931 near Beltsville, Md., and Florence, S.C. The plants were spaced 15 inches apart in rows 3 feet apart. The best plants from the first pulling from the plant bed were used; all small and inferior plants were discarded. In general, a satisfactory stand of plants was obtained from the original settings except in the 1929 planting at Beltsville. Dry weather following setting in 1929 resulted in a considerable loss of plants, but the missing hills of the entire area were replanted within 10 days of the first setting. Since weather conditions were more favorable at this time, the plants started growth more rapidly than those from the first planting, and after a few weeks there was no apparent difference in the two plantings. Little or no replanting was necessary in 1930 and 1931. The stand in all cases was about as satisfactory as could be obtained on areas as large as those used.

Throughout the growing season weeds were kept down by the usual horse cultivation and hand hoeing.

Care was exercised in harvesting to prevent the movement of the roots in the row from one 15-foot unit to another and to insure the removal of all roots from the soil. It was found necessary to remove the vines from the plants before plowing out the roots.

Records were taken for the yield for each 15-foot unit of row. The yields were recorded to the nearest quarter of a pound. All small roots were excluded from the weight records, the same standard of grading being followed as nearly as possible throughout.

PLANTING IN 1929

The first planting was made in the spring of 1929 on a piece of sandy loam containing some gravel near Beltsville, Md. The yield records were taken from an area of approximately 1 acre consisting of 50 rows 300 feet long.

The land was spring-plowed and fertilized at planting time with 600 pounds of a 2–8–10[4] fertilizer applied in the bottom of the planting ridges. The fertilizer was applied by means of a wheelbarrow type of distributor and thoroughly incorporated in the soil with a 7-tooth cultivator before the ridges were made. The ridges were thrown up at right angles to the direction of plowing.

The roots used for the growing of the plants were selected from a stock maintained by the Division of Fruit and Vegetable Crops and Diseases for a number of years and were grown near Kenilworth,

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[4] Fertilizer formulas herein used refer to nitrogen, phosphorus, and potassium in the order named.

Md., in 1928. They were bedded in manure-heated plant-growing frames under glass at the Arlington Experiment Farm, Rosslyn, Va., on April 10 and 11. The "draws" were set in the field May 15 and 16. As already mentioned, dry weather prevailed at planting time, and this resulted in some loss of plants, necessitating replanting. The precipitation during the season was below normal, and a very low yield resulted. The crop was harvested October 8 and 9.

PLANTING IN 1930

The material for the 1930 study was grown on a sandy loam soil at the Pee Dee Experiment Station (a substation of the South Carolina Agricultural Experiment Station) at Florence, S.C. This land is typical of the soil used for sweetpotato production throughout Virginia and the Carolinas. The land had been in cotton in 1929 and had received an application of 840 pounds per acre of a 3–9–3 fertilizer. Previous to planting in 1930 the area used was given 600 pounds per acre of a 3–8–9 fertilizer and planting ridges were run across the direction of plowing.

Records were taken from an area of approximately 1.6 acres consisting of 130 rows 180 feet long. The plants were set in the field on May 30, the same methods being employed as had been used at Beltsville in 1929. The slips for planting were grown from roots selected from the planting stock of the Porto Rico variety, which had been used for a number of years in cooperative work with sweetpotatoes by the United States Department of Agriculture and the Pee Dee Station. The crop was given the usual care during the season. While many parts of the country suffered from drought during the season of 1930, the sweetpotato crop at the Pee Dee Station was not injured materially. It was harvested October 22 and 23 in the same manner as in 1929, and a fair yield was obtained.

PLANTINGS IN 1931

Two lots of material were grown in 1931. The first planting was made near Beltsville, Md., on the same type of soil as in 1929. Yield records were taken from an area of approximately 2 acres, consisting of 100 rows 300 feet in length. The same stock was used for plant production as in 1929. The roots were bedded in sand in water-heated frames on April 20 and 21.

The land was spring-plowed and a cover crop of rye which had been on the land during the winter was turned under. In the spring of 1930 this land had also received a green-manure crop of rye and 750 pounds of a 2–8–10 fertilizer per acre, but on account of extreme drought during the growing season of 1930, no crop had been produced. The land was prepared for the setting of the plants in the same manner as in 1929, an application of 750 pounds of a 2–8–10 fertilizer being used. The planting ridges were run across the direction in which the land was plowed.

The plants were set in the field on May 25 and 26, and the usual care was given the crop during the growing season. The crop was harvested and the yield records taken on October 8 and 9 in the same manner as in previous years.

The second planting in 1931 from which yield data were recorded comprised slightly more than 1 acre of sandy loam soil at the Pee Dee

Station. The land had received an application[4] of 500 pounds per acre of a 4–8–4 fertilizer in the spring of 1930 and a crop of corn had been grown. Before the sweetpotatoes were planted in 1931 the area was given an application of 600 pounds of a 3–8–9 fertilizer per acre. This was the only one of the four plantings in which the rows paralleled the direction of the plowing.

The plants were set in the field on June 1, and the crop was given the usual cultivation during the season. The roots were harvested and yield records for 15-foot row units recorded November 10 and 11. The rainfall during the season was below normal, and this resulted in a low yield for land of this type in the locality of Florence.

PRESENTATION OF DATA

SIZE OF PLOT

Since four lots of material were used in making the statistical studies, it seems best to present the results for each separately and to make a final comparison of the results obtained. Each of the four lots of field data was arranged in single-row plots of various lengths and in multiple-row plots consisting of various numbers of 15-foot rows.

From the frequency distribution of yields of each size and shape of plot the mean and its probable error were computed. The standard deviation and its probable error, the error of a single determination, and the coefficient of variability were also computed.

The following formulas were employed in calculating the various measures of variability:

$$\text{Mean yield (pounds per acre)} = \frac{\Sigma\,(fV)}{n} \qquad (1)$$

$$\text{Probable error of mean} = \pm\frac{0.6745\,\sigma}{\sqrt{n}} \qquad (2)$$

$$\text{Standard deviation} = \sqrt{\frac{\Sigma\,(fd^2)}{n}} \qquad (3)$$

$$\text{Probable error of standard deviation} = \pm\frac{0.6745\,\sigma}{\sqrt{2n}} \qquad (4)$$

$$\text{Probable error of a single determination} = \pm\,0.6745\,\sigma \qquad (5)$$

$$\text{Coefficient of variability} = \frac{100\,\sigma}{M} \qquad (6)$$

The significance of differences in standard deviation for various sizes of plots was determined by the probable error of the difference. A difference of more than three times its probable error was assumed to be significant.

In assembling the field records for single-row plots, each set of records was arranged so that the entire planting was considered as a single linear row; that is, the 15-foot units were taken consecutively down one row and back the next throughout the entire area.

In forming the multiple-row plots the adjacent 15-foot units were taken as they were arranged in the field. Since the number of field rows in each planting was not divisible by the number of rows per plot for all plot sizes, there were some odd rows in certain cases. These were eliminated, so that all plots might be composed of adjacent parallel rows.

The number of plot replications required, as given in the last columns of tables 1 to 5, inclusive, was determined by formula 7 as explained under the discussion of plot replication (p. 392).

EXPERIMENTS AT BELTSVILLE, MD., 1929

A summary of the data for single-row plots from the planting made near Beltsville in 1929 is presented in table 1. One thousand single-row units 15 feet in length were available for study. Frequencies were formed for single-row plots of 15, 30, 45, 60, 75, 90, 120, 150, and 180 feet. By increasing the size of a single-row plot from 15 feet to 180 feet the error of a single determination was reduced 515 pounds per acre, 55.7 percent of which resulted from increasing the size from 15 feet to 60 feet, 70.9 percent from increasing it from 15 feet to 75 feet, and 82.7 percent from increasing it from 15 feet to 90 feet. The relation of the curves for the actual and theoretical standard deviations is shown in figure 1.

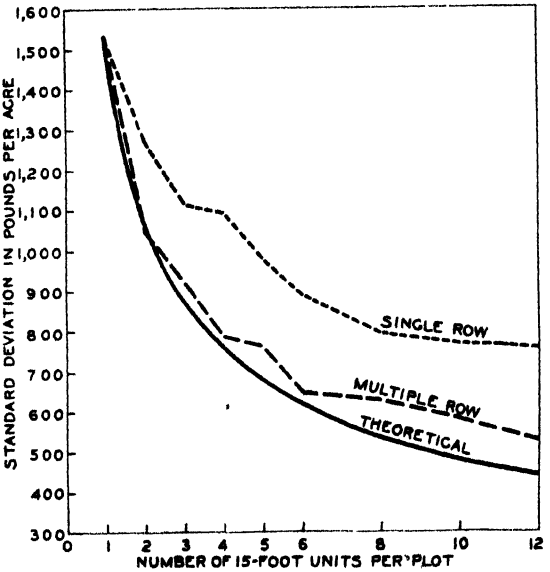

FIGURE 1 —Actual and theoretical deviations in yields for single-row and multiple-row plots of sweetpotatoes at Beltsville, Md., 1929.

The significance of differences in standard deviation for the various plot sizes is shown in figure 2, *A*. Significant differences in standard deviation were found between row lengths of 15 feet and 30 feet, 30 feet and 45 feet, 45 feet and 75 feet, and between 75 feet and 120 feet. Increasing the row length from 120 feet to 180 feet did not give a significant difference in standard deviation. No significance was found between rows of 90 feet and greater length of row up to 180 feet.

It is obvious even as a purely mathematical consideration that when a unit is replicated or increased by the amount of itself the point is soon reached where the addition of another unit has little influence on the standard deviation, since the first replication increases the whole by 100 percent, the second by 50 percent, the third by 33.33 percent, the fourth by 25 percent, and so on, each additional replication having less influence on the standard deviation. In experiments of this kind numerous varying factors enter which prevent the curve of actual standard deviations from coinciding with the theoretical curve. Where a large number of varieties or strains are to be compared, numerous replications of each may require so large an area that the soil variation encountered may be very great. In such instances a smaller number of replications requiring less land might give a smaller standard deviation than a larger number of replications.

The one thousand 15-foot row units in the 1929 planting were arranged in multiple-row plots which included 1, 2, 3, 4, 5, 6, 8, 10,

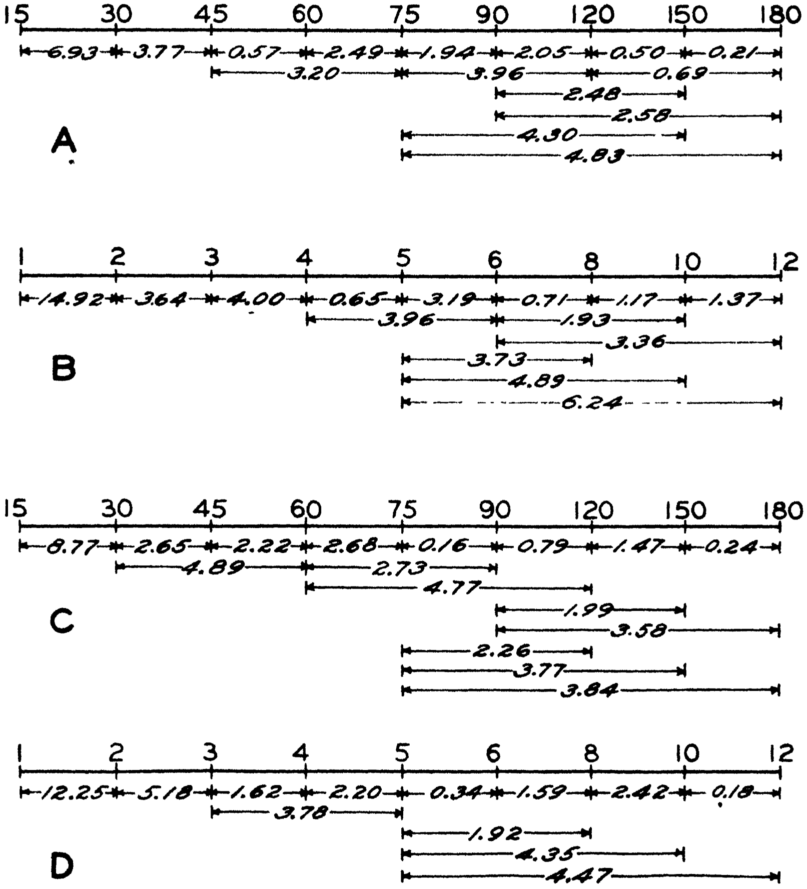

FIGURE 2 - Comparisons showing significance of reduction of standard deviation resulting from increase in size of plots of sweetpotatoes *A*, Single-row plots, and *B*, multiple-row plots. Beltsville, Md., 1929; *C*, single-row plots, and *D*, multiple-row plots, Florence, S.C., 1930 Upper row of figures in *A* and *C* indicate length of row per single-row plot, upper row of figures in *B* and *D* indicate number of rows per multiple-row plot, with individual rows 15 feet long Other figures represent coefficients of odds for differences between standard deviations for the plot sizes indicated

and 12 adjacent 15-foot row units. A summary of the data for the multiple-row plots from this material is shown in table 1.

The error of a single determination was reduced 671 pounds per acre by increasing the plot size from one 15-foot row to twelve 15-foot rows. Of this reduction in error of a single determination 73.8 percent was found to be the result of increasing the number of 15-foot rows per plot from 1 to 4. Increasing the number of rows per plot to 5 produced 76.1 percent of the reduction and the 6-row plot produced 87.5 percent.

TABLE 1.—*Summary of data for single-row and multiple-row plots of sweetpotatoes at Beltsville, Md., 1929*

Number of 15-foot row units per plot	Plots		Mean yield per acre		Probable error of mean		Standard deviation		Probable error of standard deviation	
	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row
	Number	*Number*	*Pounds*	*Pounds*						
1	1,000	1,000	4,687.5	4,687.5	±32.37	±32.37	1,517.5	1,517.5	±22.88	±22.88
2	500	500	4,664.0	4,660.0	±38.25	±31.42	1,268.0	1,041.5	±27.05	±22.22
3	333	320	4,641.0	4,587.5	±41.30	±34.72	1,117.5	921.0	±29.20	±24.55
4	250	240	4,668.0	4,681.0	±46.61	±34.12	1,092.5	783.5	±32.96	±24.12
5	200	200	4,667.5	4,630.0	±46.58	±36.03	976.5	760.5	±32.93	±25.65
6	166	160	4,641.5	4,681.0	±46.42	±34.53	886.5	647.5	±32.82	±24.41
8	125	120	4,646.0	4,679.0	±47.66	±38.28	790.0	621.5	±33.70	±27.06
10	100	100	4,665.0	4,655.0	±51.60	±38.88	765.0	576.5	±36.49	±27.50
12	83	80	4,614.5	4,637.5	±55.83	±39.46	754.0	523.0	±39.49	±27.88

Number of 15-foot row units per plot	Probable error of a single determination		Coefficient of variability		Plot replications necessary to meet the requirements of formula 7			
					Single row		Multiple row	
	Single row	Multiple row	Single row	Multiple row	c=20 percent	c=10 percent	c=20 percent	c=10 percent
					Number	*Number*	*Number*	*Number*
1	1,023.6	1,023.6	32.37	32.37	18.06	72.25	18.06	72.25
2	855.3	702.5	27.20	22.35	12.71	50.41	8.58	34.22
3	753.8	621.2	24.10	20.08	9.98	39.69	6.92	29.70
4	736.9	528.5	23.40	16.74	9.42	37.45	4.84	19.18
5	658.7	513.0	20.90	16.43	7.51	29.92	4.66	18.49
6	597.9	436.7	19.10	13.83	6.25	25.00	3.31	13.10
8	532.9	419.2	17.00	13.28	4.97	19.80	3.06	12.11
10	516.0	388.9	16.40	12.38	4.62	18.40	2.62	10.50
12	508.6	352.8	16.30	11.28	4.58	18.23	2.19	8.70

TABLE 2.—*Summary of data for single-row and multiple-row plots of sweetpotatoes at Florence, S.C., 1930*

Number of 15-foot row units per plot	Plots		Mean yield per acre		Probable error of mean		Standard deviation		Probable error of standard deviation	
	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row
	Number	*Number*	*Pounds*	*Pounds*						
1	1,560	1,560	11,422.5	11,422.5	±39.89	±39.84	2,335.0	2,335.0	±28.80	±28.20
2	780	780	11,542.9	11,[illegible]36.5	±47.14	±43.38	1,952.0	1,796.0	±33.33	±30.67
3	520	516	11,437.5	11,526.0	±51.50	±46.30	1,817.9	1,559.3	±38.02	±33.79
4	390	384	11,462.8	11,536.5	±57.85	±50.91	1,694.0	1,479.0	±40.91	±36.00
5	312	260	11,419.9	11,502.0	±58.72	±50.70	1,537.5	1,360.0	±41.51	±40.23
6	260	252	11,457.8	11,502.0	±63.90	±56.97	1,527.5	1,340.5	±45.19	±40.27
8	195	192	11,428.2	11,510.6	±67.38	±60.68	1,394.6	1,246.5	±47.60	±42.90
10	156	130	11,419.9	11,434.5	±69.88	±64.76	1,294.0	1,094.5	±49.42	±45.80
12	130	120	11,407.7	11,512.5	±74.99	±66.68	1,276.5	1,082.5	±53.70	±47.14

Number of 15-foot row units per plot	Probable error of a single determination		Coefficient of variability		Plot replications necessary to meet the requirements of formula 7			
					Single row		Multiple row	
	Single row	Multiple row	Single row	Multiple row	c=20 percent	c=10 percent	c=20 percent	c=10 percent
					Number	*Number*	*Number*	*Number*
1	1,575.8	1,575.8	20.44	20.44	7.2	28.62	7.20	28.62
2	1,316.6	1,211.4	16.91	15.57	4.94	19.62	4.20	16.70
3	1,226.1	1,051.7	15.89	13.53	4.36	17.30	3.13	12.44
4	1,142.6	997.6	14.78	12.82	3.76	14.98	2.82	11.20
5	1,037.0	917.3	13.46	11.82	3.13	12.39	2.40	9.84
6	1,030.3	904.2	13.33	11.60	3.06	12.18	2.31	9.18
8	940.6	840.8	12.20	10.83	2.56	10.18	2.02	8.03
10	872.8	738.2	11.33	9.57	2.22	8.82	1.56	6.20
12	854.9	730.2	11.11	9.40	2.15	8.47	1.51	6.00

The curves in figure 1 show the relation of the actual and theoretical standard deviation for the various single-row and multiple-row plots.

Significant differences in standard deviations for the various-sized plots are shown in figure 2, *B*. Significant differences were found between plots of 1 row and 2 rows, 2 rows and 3 rows, 3 rows and 4 rows, 4 rows and 6 rows, and between 6 rows and 12 rows.

EXPERIMENTS AT FLORENCE, S.C., 1930

One thousand five hundred and sixty 15-foot single-row units were available from the planting at Florence in 1930. The data for single-row plots are summarized in table 2. Increasing the length of row per plot from 15 feet to 180 feet resulted in a reduction of the error of a single determination of 721 pounds per acre, 60.1 percent of which resulted from increasing the row length from 15 feet to 60 feet, 74.7 percent from increasing it to 75 feet, and 75.6 percent from increasing it to 90 feet.

Figure 3 shows the curves of the actual and theoretical reduction in the standard deviation resulting from increases in row length of single-row plots.

The significance of differences in standard deviations of various row lengths is shown in figure 2, *C*. Significant differences were found between row lengths of 15 feet and 30 feet, 30 feet and 60 feet, and 60 feet and 120 feet. Increasing the row length above 120 feet gave no significant differences.

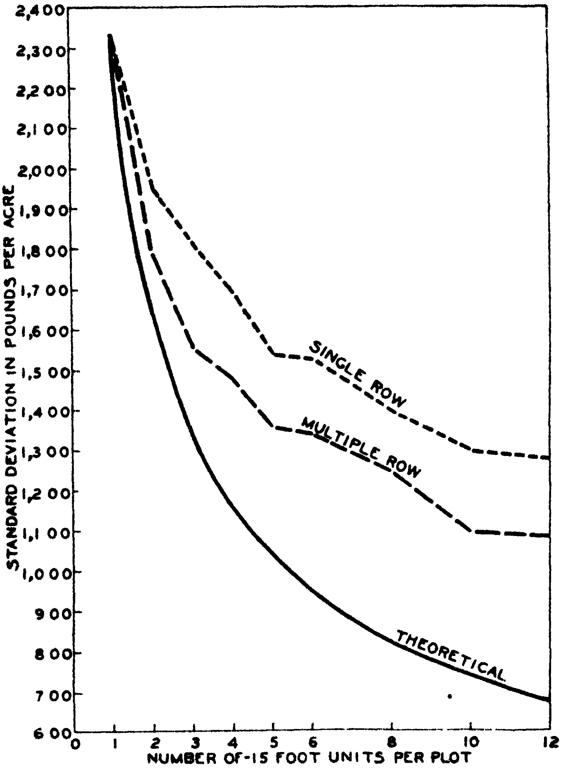

FIGURE 3.—Actual and theoretical deviations in yields for single-row and multiple-row plots of sweetpotatoes, Florence, S.C., 1930

The summary of the data resulting from assembling one thousand five hundred and sixty 15-foot row units into multiple-row plots in table 2 shows the error of a single determination to have been reduced 846 pounds per acre by increasing the number of 15-foot rows per plot from 1 to 12. Increasing the number of rows to 4 produced 68.4 percent, 5 rows produced 77.9 percent, and 6 rows produced 79.4 percent of the total reduction in the error of a single determination.

The standard deviation curves are shown in figure 3.

As indicated in figure 2, *D*, significant differences in standard deviation were found between 1 row and 2 rows, 2 rows and 3 rows, and 3 rows and 5 rows. Above 5 no significant difference in standard deviation was found until the number of 15-foot rows per plot was increased to 10.

EXPERIMENTS AT BELTSVILLE, MD., 1931

The planting made near Beltsville in 1931 gave two thousand 15-foot row units. By increasing the single-row plots from 15 feet to 180 feet, the error of a single determination for single-row plots was reduced from 1,603 pounds for a 15-foot row to 886 pounds for a 180-foot row, or a reduction of 717 pounds (table 3). Increasing the row length from 15 feet to 60 feet produced 61.3 percent of the reduction; the 75-foot and 90-foot plots produced 74.2 and 82 percent, respectively, of the total reduction.

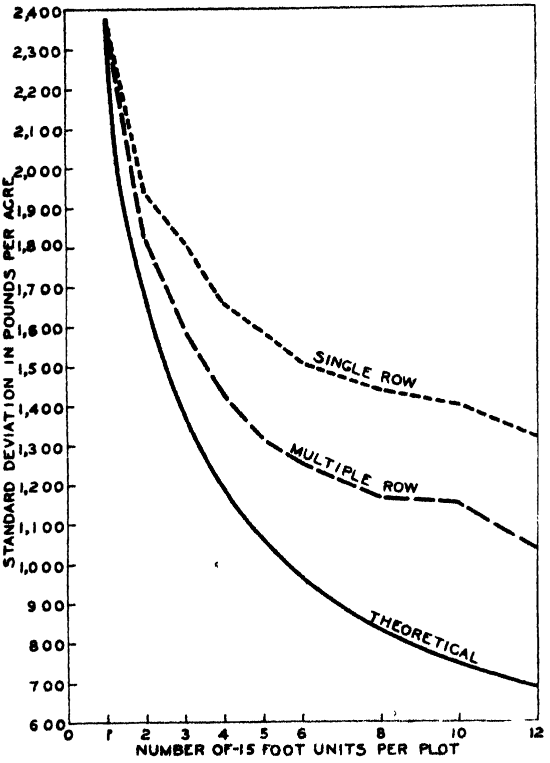

FIGURE 4.—Actual and theoretical deviations in yields for single-row and multiple-row plots of sweetpotatoes, Beltsville, Md., 1931.

Figure 4 shows the relation of the standard deviation curves.

As shown by figure 5, A, significant differences in standard deviation were found between row lengths of 15 feet and 30 feet, 30 feet and 60 feet, and 60 feet and 120 feet.

When this material was assembled in multiple-row plots of varying numbers of 15-foot rows, as shown in table 3, a reduction of 906 pounds per acre in the error of a single determination resulted from increasing the number of 15-foot rows per plot from 1 to 12. Of the 906 pounds, 70.5 percent resulted from increasing the number of rows from 1 to 4, 78.8 percent from increasing it from 1 to 5, and 83.8 percent from increasing it from 1 to 6.

The standard deviation curves for multiple-row plots are shown in figure 4 and the significance of differences in standard deviation in figure 5, *B*. Significant differences were found between plots of 1 row and 2 rows, 2 rows and 3 rows, 3 rows and 4 rows, and 4 rows and 6 rows. Increasing the number of rows above 6 did not give a significant difference in standard deviation until the number of rows was increased to 12.

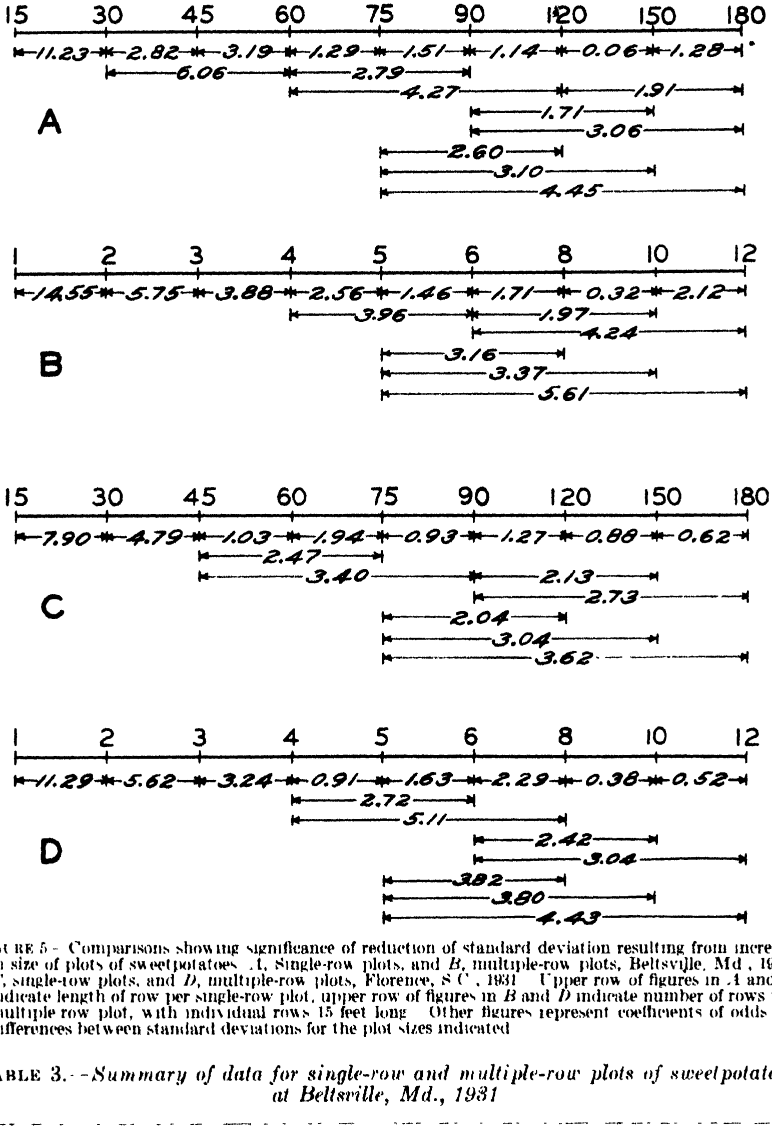

FIGURE 5.—Comparisons showing significance of reduction of standard deviation resulting from increase in size of plots of sweetpotatoes *A*, Single-row plots, and *B*, multiple-row plots, Beltsville, Md., 1931, *C*, single-row plots, and *D*, multiple-row plots, Florence, S.C., 1931. Upper row of figures in *A* and *C* indicate length of row per single-row plot, upper row of figures in *B* and *D* indicate number of rows per multiple row plot, with individual rows 15 feet long. Other figures represent coefficients of odds for differences between standard deviations for the plot sizes indicated.

TABLE 3.—*Summary of data for single-row and multiple-row plots of sweetpotatoes at Beltsville, Md., 1931*

Number of 15-foot row units per plot	Plots		Mean yield per acre		Probable error of mean		Standard deviation		Probable error of standard deviation	
	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row
	Number	*Number*	*Pounds*	*Pounds*						
1	2,000	2,000	7,741.0	7,741.0	±35.84	±35.84	2,376.5	2,376.5	±25.75	±25.75
2	1,000	1,000	7,842.5	7,888.5	±41.36	±38.98	1,939.0	1,827.5	±29.25	±27.56
3	666	660	7,873.0	7,880.0	±47.39	±41.88	1,813.5	1,595.0	±33.52	±29.61
4	500	500	7,846.0	7,857.0	±49.92	±43.14	1,655.0	1,430.0	±36.78	±30.50
5	400	400	7,877.5	7,826.2	±53.52	±44.45	1,587.0	1,318.0	±37.84	±31.44
6	333	320	7,841.0	7,839.0	±55.60	±47.17	1,504.5	1,251.0	±39.32	±33.35
8	250	240	7,788.0	7,800.0	±61.33	±50.82	1,437.5	1,167.0	±43.36	±35.92
10	200	200	7,914.5	7,850.0	±66.52	±54.86	1,399.5	1,150.0	±47.20	±38.78
12	166	160	7,831.5	7,850.0	±68.76	±55.11	1,313.0	1,033.5	±48.61	±38.96

TABLE 3.—*Summary of data for single-row and multiple-row plots of sweetpotatoes at Beltsville, Md., 1931*—Continued

Number of 15-foot row units per plot	Probable error of a single determination		Coefficient of variability		Plot replications necessary to meet the requirements of formula 7			
					Single row		Multiple row	
	Single row	Multiple row	Single row	Multiple row	c=20 percent	c=10 percent	c=20 percent	c=10 percent
					Number	*Number*	*Number*	*Number*
1	1,603.0	1,603.0	30.70	30.70	16.24	64.48	16.24	64.48
2	1,307.9	1,232.7	24.72	23.17	10.56	41.86	9.24	36.72
3	1,223.2	1,075.8	23.00	20.24	9.15	36.24	7.07	28.09
4	1,163.0	964.5	21.10	18.20	7.68	30.47	5.71	22.66
5	1,070.4	889.0	20.14	16.84	7.00	27.77	4.88	19.45
6	1,014.8	843.8	19.19	15.96	6.35	25.20	4.41	17.45
8	969.6	787.1	18.46	14.96	5.88	23.33	3.86	15.37
10	944.0	775.7	17.68	14.65	5.38	21.44	3.70	14.75
12	885.6	697.1	16.76	13.16	4.84	19.27	2.99	11.90

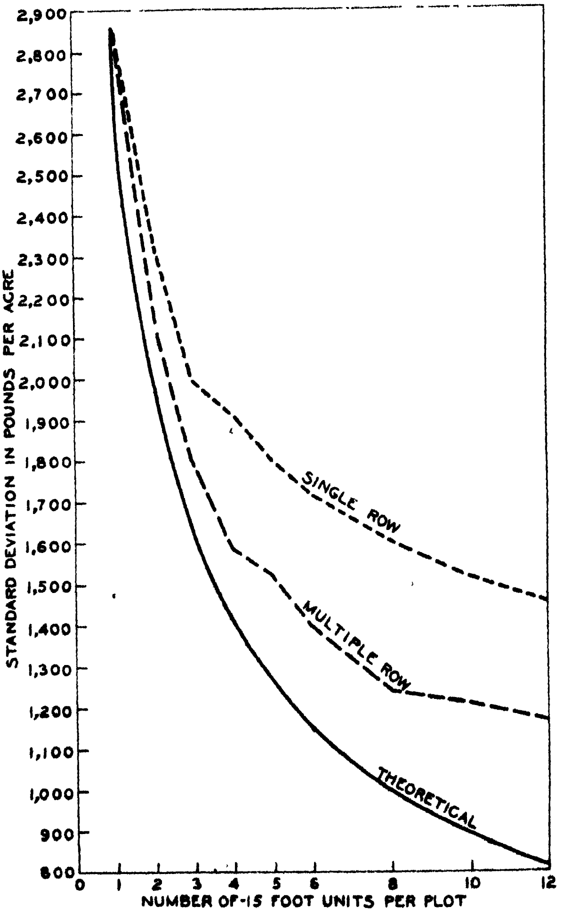

FIGURE. 6.—Actual and theoretical deviations in yields for single-row and multiple-row plots of sweetpotatoes, Florence, S C., 1931.

EXPERIMENTS AT FLORENCE, S.C., 1931

The second lot of yield records was taken in 1931 from the planting at Florence. The rows were 675 feet long. In assembling the data for the single-row plots, 23 of the 675-foot rows were used, which gave one thousand and thirty-five 15-foot units. In order to make better use of the material in assembling the multiple-row plots, one more field row was added, making one thousand and eighty 15-foot units.

The summary of data for single-row plots in table 4 shows a reduction of 951 pounds per acre in the error of a single determination as a result of increasing the row length from 15 feet to 180 feet. Increasing the row length from 15 feet to 60 feet produced 67.2 percent of the total reduction; increasing it to 75 feet produced 72.2 percent, and increasing it to 90 feet produced 81.1 percent reduction.

Figure 6 shows the standard deviation curves for the single-row and multiple-row plots.

Differences in standard deviation for various sizes of single-row plots as shown in figure 5, *C*, were found between row lengths of 15 feet and 30 feet, 30 feet and 45 feet, and 45 and 90 feet. Increasing the row length above 90 feet gave no significant differences.

TABLE 4.—*Summary of data for single-row and multiple-row plots of sweetpotatoes at Florence, S.C., 1931*

Number of 15-foot row units per plot	Plots		Mean yield per acre		Probable error of mean		Standard deviation		Probable error of standard deviation	
	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row	Single row	Multiple row
	Number	*Number*	*Pounds*	*Pounds*						
1	1,035	1,080	8,105.0	8,089.5	±61.94	±58.02	2,862.5	2,826.5	±42.58	±41.03
2	517	540	8,138.0	8,159.0	±69.39	±62.30	2,339.5	2,146.5	±50.65	±44.06
3	345	360	8,058.0	8,125.0	±72.44	±63.73	1,994.5	1,792.5	±51.21	±45.06
4	258	270	8,112.5	8,100.0	±80.67	±65.03	1,916.0	1,584.0	±56.89	±45.97
5	207	180	8,091.5	8,055.5	±84.11	±76.39	1,800.5	1,519.5	±59.69	±54.03
6	172	180	8,032.0	8,119.5	±88.46	±70.36	1,720.0	1,400.0	±62.55	±49.78
8	129	135	8,019.5	8,059.0	±95.22	±71.98	1,603.5	1,240.0	±67.34	±49.09
10	103	90	8,102.0	8,032.0	±101.83	±86.03	1,517.5	1,210.0	±71.33	±60.83
12	86	90	8,017.5	8,088.5	±105.76	±82.91	1,453.5	1,166.0	±74.75	±58.62

Number of 15-foot row units per plot	Probable error of a single determination		Coefficient of variability		Plot replications necessary to meet the requirements of formula 7			
					Single row		Multiple row	
	Single row	Multiple row	Single row	Multiple row	c=20 percent	c=10 percent	c=20 percent	c=10 percent
					Number	*Number*	*Number*	*Number*
1	1,930.8	1,906.5	35.32	34.94	21.52	85.56	21.07	83.72
2	1,578.0	1,447.8	28.75	26.31	14.21	56.70	11.90	47.47
3	1,345.3	1,209.0	24.75	22.06	10.56	41.99	8.35	33.41
4	1,292.3	1,068.4	23.62	19.55	9.61	38.19	6.60	26.21
5	1,244.4	1,024.9	22.25	18.86	8.52	33.99	6.10	21.40
6	1,160.1	944.3	21.41	17.24	7.89	31.36	5.10	20.34
8	1,081.6	836.4	19.99	15.39	6.86	27.35	4.08	16.24
10	1,023.6	818.1	18.73	15.08	6.05	24.01	3.92	15.60
12	980.0	786.5	18.13	14.42	5.66	22.56	3.57	14.29

The one thousand and eighty 15-foot units used in the data as summarized in table 4 show a reduction in the error of a single determination of 1,120 pounds per acre resulting from increasing the number of 15-foot rows per plot from 1 to 12. Of the total reduction 74.8 percent was between 1 and 4 rows per plot, 78.7 percent between 1 and 5 rows per plot, and 85.9 percent between 1 and 6 rows per plot.

The curves of standard deviations are shown in figure 6. The differences in standard deviations for various sized plots as indicated in figure 5, *D*, show significance between plots of 1 row and 2 rows, 2 rows and 3 rows, 3 rows and 4 rows, and between 5 rows and 8 rows.

DETERMINATION OF NUMBER OF PLOT REPLICATIONS REQUIRED

Various mathematical formulas have been derived for calculating the number of replications, or number of samples distributed at random, that must be used in order that a certain difference between two averages may be considered significant. The formula derived by Mitchell and Grindley (*7*) and applied to field-plot studies, notably by Anthony and Waring (*1*) in analyzing yield records from fertilizer experiments with apple trees, was used in this study. The value for N is determined by the formula

$$N=\left[\frac{1.849\ C\sqrt{2\frac{1}{2}c^2}}{100\ c}\right]^2 \qquad (7),$$

in which N is the least number that can be used when a percentage difference of c in yields between samples and odds of 30 to 1 is desired with material showing a coefficient of variability of C. The value for N as calculated by the formula can only be the most probable value, since its determination is dependent upon the coefficient of variability and must vary with the probable error of C.

The values for N as determined by the foregoing formula for the various sizes of single-row and multiple-row plots are given as the last four columns in tables 1 to 4, inclusive. The N values for the various plot shapes are given as the last items in table 5. The required number of replications has been determined for 10 and 20 percent difference for the value c in the formula.

TABLE 5.—*Summary of data on shape of plots of sweetpotatoes at Beltsville, Md., 1929 and 1931, and at Florence, S.C., 1930 and 1931*

Location and year	Rows per plot		Number of plots	Mean yield (pounds per acre)	Probable error of mean	Standard deviation	Probable error of standard deviation	Probable error of a single determination	Coefficient of variability	Number of plot replications necessary to meet the requirements of formula 7	
	Number	Length (feet)								$c=20$ percent	$c=10$ percent
Beltsville, Md., 1929	1	90	166	4,641.5	±46.42	886.5	±32.82	597.9	19.1	6.25	25.00
	2	45	166	4,671.5	±48.07	918.0	±33.98	619.2	19.65	6.66	26.42
	3	30	160	4,656.0	±45.82	859.3	±32.40	579.6	18.43	5.86	23.23
	6	15	160	4,681.0	±34.53	647.5	±24.41	436.7	13.83	3.31	13.10
Florence, S.C., 1930	1	90	260	11,457.0	±63.90	1,527.5	±45.19	1,030.3	13.33	3.06	12.18
	2	45	260	11,500.0	±61.91	1,480.6	±43.80	998.63	12.87	2.86	11.36
	3	30	258	11,469.0	±56.61	1,347.9	±40.03	909.2	11.75	2.37	9.48
	6	15	260	11,502.0	±56.97	1,340.5	±40.27	904.8	11.60	2.31	9.18
Beltsville, Md., 1931	1	90	333	7,841.0	±55.60	1,504.5	±39.32	1,014.8	19.19	6.35	25.20
	2	45	333	7,837.8	±52.45	1,419.2	±37.10	957.2	18.11	5.62	22.47
	3	30	330	7,860.5	±51.75	1,393.8	±36.59	940.1	17.73	5.42	21.53
	6	15	320	7,839.0	±47.17	1,251.0	±33.35	843.8	15.96	4.41	17.47
Florence, S.C., 1931	1	90	172	8,032.0	±88.46	1,720.0	±62.55	1,160.1	21.41	7.89	31.36
	2	45	180	8,077.8	±84.32	1,677.3	±59.63	1,131.3	20.76	7.45	29.48
	3	30	176	8,073.9	±73.27	1,441.1	±51.81	972.0	17.85	5.47	21.81
	6	15	180	8,119.5	±70.36	1,400.0	±49.78	944.3	17.24	5.10	20.34

INFLUENCE OF SHAPE AND ORIENTATION OF PLOTS ON VARIATION

In order to study the effect of shape of plot and its orientation in relation to the field rows on variation among plots, each of the four lots of material was arranged in four different plot shapes, each consisting of 90 linear feet of row.

The 90-foot row was chosen for two reasons: (1) It permits a satisfactory arrangement of the 15-foot units into the various shapes of plots containing the same number of linear feet of row; (2) the data (figs. 2 and 5) indicated that little significant reduction in plot variability can be obtained by the use of a plot of more than 90 linear feet of row, hence it is desirable that the study be made within the limits of the largest plot shown to give a significant difference in standard deviation.

The four groups of equal plots consisted of one 90-foot row approximately 3 by 90 feet, plots of two 45-foot rows approximately 6 by 45

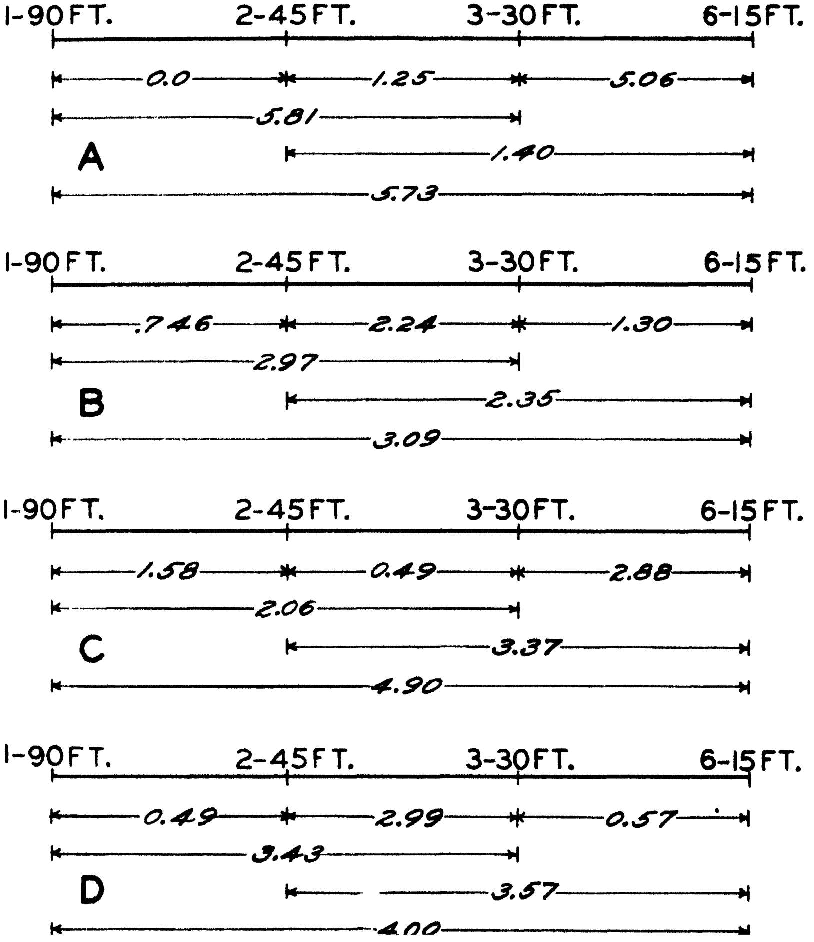

FIGURE 7. Comparisons showing significance of standard deviations for 4 shapes of plots of sweetpotatoes. *A*, Beltsville, Md., 1929, *B*, Florence, S.C., 1930, *C*, Beltsville, 1931, *D*, Florence, 1931. Plots of equal area. Upper row of figures in each group show number and length of rows per plot.

feet, plots of three 30-foot rows approximately 9 by 30 feet, and plots of six 15-foot rows approximately 18 by 15 feet.

A summary of the data for the various-shaped plots for the four lots of material is presented in table 5.

Figure 7 shows the significance of differences in standard deviation between the various shapes of plots. Figure 8 presents a graphic comparison of the standard deviations for the various plot shapes for the four lots of data.

SOIL HETEROGENEITY AND VARIABILITY

The variability of the soil in the four pieces of land used for this work was determined in each case by Harris' method (*4, ch. 4*), in which the coefficient of correlation is used as an index of soil uniformity.

In applying the Harris method each field was divided into small ultimate units consisting of two rows 75 feet long. The yield from each unit was determined in pounds per acre. Groups were formed of four adjacent units. The correlation between the ultimate units and the groups was determined by the following formula:

$$r_{p_1 p_2} = \frac{\{[S(Cp^2) - S(p^2)]/m[n(n-1)]\} - p^2}{\sigma p^2} \qquad (8),$$

in which—

p = Average yield of all ultimate units.
n = Number of units in each group.
m = Number of groups.
$S(p^2)$ = Sum of squares of yields of ultimate units.
$S(Cp^2)$ = Sum of squares of the group yields.
σp = Standard deviation of the yields of ultimate units.

The constant obtained is used as a measure of the correlation between adjacent plots when grouped in a particular way.

If certain adjacent units tend to yield high and others low, a large coefficient is obtained, indicating that the field is "patchy"; a small coefficient indicates that the variation is largely due to random sampling. If the variation is due only to random sampling, the correspondence between certain contiguous units will be balanced by a lack of correspondence between others, provided the number of units is large enough to permit the expression of the law of averages.

The values in the formula for each of the four pieces of land employed in these studies are given in table 6.

TABLE 6.—*Summary of data on soil heterogeneity for the 4 areas of land used in the sweetpotato-plot studies*

[Size of ultimate units, two 75-foot rows; 4 units in each group]

Source of data	Groups	Average yield of ultimate units per acre	Sum of squares of ultimate units	Sum of squares of group yields	Standard deviation of yields of ultimate units	Coefficient of of correlation
	Number	*Pounds*				
Beltsville, Md., 1929	25	4,643.6	2,212,797,177	8,730,059,616	635.5	0.4000±0.0566
Florence, S.C., 1930	39	11,428.0	20,618,285,000	82,125,080,000	1,239.6	.5372± .0384
Beltsville, Md., 1931	50	7,833.0	12,611,195,000	49,780,610,000	1,304.0	.3488± .0419
Florence, S.C., 1931	27	8,051.2	7,222,585,625	28,477,223,525	1,420.0	.3863± .0552

The area used at Florence in 1930 was found to be the most variable, the coefficient being 0.5372 ± 0.0384. The most uniform of the four pieces was that used at Beltsville in 1931, the value for *r* being 0.3488 ± 0.0419. The areas used at Beltsville in 1929 and at Florence in 1931 gave correlation coefficients of 0.4000 ± 0.0566 and 0.3863 ± 0.0552, respectively.

The sources of variation other than soil heterogeneity which may influence the results of an experiment of this kind are numerous.

Where more than one thousand 15-foot units are dug, sorted, and weighed, the possibility for errors is much greater than would be the case in an actual experiment if the size of plot and number of replications recommended were followed.

Even with the best of methods it is not possible to run a large number of rows that will be exactly the same distance apart throughout their entire length; in some cases a difference of a few inches may be important. Where the plots are long and narrow and parallel the direction of the rows it is particularly important that the distances between rows be uniform.

There is always some variation in the size and vigor of plants from different parts of the plant-growing beds, especially when the heat is supplied by animal manure. Manure is quite variable in composition and heating qualities. Even in water-heated frames there may be considerable difference in growth resulting from variations in temperature. If the beds are long, the part of the frame that first receives the water from the boiler may be several degrees warmer than the end farthest from the boiler.

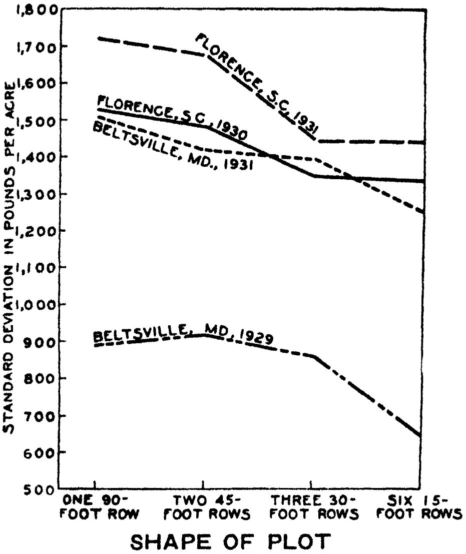

FIGURE 8.—Comparison of standard deviation curves for sweetpotato plots of equal area and varying shape

Plants are generally pulled and handled in such a way that a large part of one row may be set with the better plants while the next may be set with smaller and less vigorous ones. Where several setters do the work, the variations resulting from methods of setting may be considerable. Rain at the time the plants are being set may also cause variation. The amount of variation from this and other sources in field tests is difficult to determine.

It should be noted that the variations in plot yields for the various areas of land as indicated by the coefficients of variation given in tables 1 to 4 do not coincide with the variations in soil indicated by the coefficients of correlation in table 6; that is, the greatest variation in plot yields as indicated by the coefficients of variation did not occur on the land shown by the Harris method to be the most heterogeneous. The most heterogeneous piece of land—that used at Florence, in 1930, with a coefficient of correlation of 0.5372 ± 0.0384 (table 6)—gave the most uniform yields, as indicated by the coefficients of variation in tables 2 and 3. The land used at Florence in 1931 ranked second in soil uniformity, as indicated by the coefficient of correlation, table 6, but gave the most variable yields, as indicated by the coefficients of variation in tables 4 and 5.

The lack of correlation between soil heterogeneity and variation in yields indicates that some of the other factors mentioned contributed materially to the variation in yields between plots. Since insufficient records were made as to the location of groups of large and small plants, the portions of rows planted by the several setters, and the variations in width of spacing between rows, it was not possible to determine the relative importance of these factors.

The greater uniformity of yields from multiple-row plots as compared with yields from single-row plots is evidence that variation existed between adjacent rows. Since it is not likely that the rows were run parallel to soil gradients in every case, and in 3 of the 4 fields the rows were run across the direction of the plowing so that variation due to dead furrows was minimized in these 3 fields, it is evident that much of the variation between rows was due to factors other than soil heterogeneity.

DISCUSSION

SIZE OF PLOT

An analysis of the data in figures 2 and 5 reveals the fact that in only one of the eight studies presented was there a significant difference in standard deviation between the various sizes of plots obtained by increasing the size of plot above 90 linear feet of row until the plot contained 180 linear feet. In the multiple-row plots of the 1930 material a significant difference was found between the 90-foot and the 150-foot plots. In every case significant differences in standard deviation were noted between plots containing 75 linear feet of row and those containing 180 feet of row. In 4 of these, significant differences were found between plots of 75 and 120 linear feet of row; in the other 4, significant differences were found between plots of 75 and 150 feet of row.

It is concluded from these data that a significant reduction in variation may result from increasing the size of plot until it includes about 90 linear feet of row. Further increase in size of the area would be of doubtful value in reducing variability between plots in field experiments with sweetpotatoes.

SHAPE AND ORIENTATION OF PLOT

The data presented in figure 7 show a significant difference in standard deviation between the 90-foot single-row plots and the plots of six 15-foot adjacent rows, in all four cases in favor of the latter. Between the other plots there was no consistent difference. The effect of row grouping in reducing variation is shown graphically in figures 1, 3, 4, and 6.

While regression curves for soil variation were not calculated and the direction of the greatest soil variation was not determined, it is not likely that in every case the rows were run parallel to such soil gradients as might have been present. Yet there was a significant reduction in the standard deviation in each case between long narrow single-row plots and almost square multiple-row plots. It seems safe to assume that much of the reduction in variation in favor of the square plots was due to the number of rows included within the plots, indicating that variation existed between rows and was probably due to factors other than soil heterogeneity.

If an appreciable amount of the variation among plots is due to correlated variations between different rows, it is obvious that plots which include portions of several rows are likely to show less variation than plots consisting of a single row. Variation between rows resulting from methods used by plant setters can be controlled to better advantage in actual field-plot tests where the plots are set as units than where the entire area is planted as a unit, as was practiced throughout this experiment. By having each setter plant an equal portion of each plot, much of the variation from this source can be eliminated. Variation due to difference in spacing of rows can be minimized by the careful laying out of rows but cannot be entirely avoided.

Previous experiments on methods of eliminating variation in field-plot tests in most cases have indicated that there is little difference in favor of either square or long narrow plots. Where a difference has been found, it has been in favor of the latter. However, Day (*3*) points out that such plots are superior to square ones only when the longest dimension parallels the direction of greatest soil variation. Much of the work on the shape of plots has been conducted with small grains or other crops that are planted by drill, thereby eliminating some of the sources of variation that enter into transplanted crops such as the sweetpotato.

From the results of the four lots of material studied in this experiment it is concluded that square plots are superior to long narrow ones for sweetpotato field-plot tests, unless such plots are run across the field rows so as to include portions of several rows. Long narrow plots would not be the most economical of land where border rows are necessary, since the perimeter is much greater than that of square plots.

REPLICATION

The large number of replications required where significance is desired in a percentage difference of less than 20 indicates that it would not be safe to attach importance to differences of much less than 20 percent for a single year's data in field-plot tests with sweetpotatoes where any large number of treatments are to be compared. When investigations are conducted over a period of years the accumulated number of replications may be sufficient for the determination of significance of differences as small as 10 percent. The data must, however, be treated by Student's (*5*) or some other recognized method to remove correlation attributable to seasonal conditions where the results obtained in consecutive years are to be compared.

The number of replications required to give a desired degree of accuracy decreases as the size of the plot is increased within the limits stated in the study. Since analyses of the data in figures 2 and 5 show that the reduction in variation brought about by increasing the size of plot above 90 linear feet of row is of doubtful significance, it would not be economical to use plots consisting of much more than about 90 linear feet of row where the rows are spaced 3 feet apart. Even though significant reduction in variation may be obtained by the use of plots containing more than 90 linear feet of row, it is not economical of land to use the largest plot found to give significant reduction in variability.

In some cases the area of uniform land available for experimental work may be a limiting factor. The values for N as determined by the Mitchell and Grindley formula (*7*) indicate that the same degree of accuracy can be obtained by using small plots as by using large ones, with significant economy in land even though a much larger number of replications is required. For example, take the multiple-row plots of the data from the 1931 planting at Florence, which showed the greatest variation as indicated by the coefficient of variability. By employing the formula derived by Mitchell and Grindley and using a value of 20 percent for *c*, the number of replications and the total linear feet of row required by the various sizes of plots are found to be, for a single 15-foot row plot, 21 replications or 315 feet of row; for a plot of two 15-foot rows, 11.9 replications or 357 feet of row; for a plot of three 15-foot rows, 8.35 replications or 376 feet of row; for a plot of five 15-foot rows, 6.1 replications or 458 feet of row; for a plot of six 15-foot rows, 5.1 replications or 459 feet of row; for a plot of eight 15-foot rows, 4 replications or 480 feet of row; for a plot of ten 15-foot rows, 3.9 replications or 585 feet of row; and for a plot of twelve 15-foot rows, 3.6 replications or 648 feet of row. Fractions of replications are used here only for the purpose of comparison. For this lot of material there is a saving in land of 9.8 percent between two and four 15-foot rows, and 11.5 percent between four and six 15-foot rows. A similar saving in land by the use of small plots is shown in all four lots of data. In some cases the saving in land is much greater. The use of very small plots has the practical disadvantage of additional expense and labor in planting and harvesting the crop; in fact the use of very small plots may result in more expense and labor than the other advantages justify. Where guard rows are necessary small plots may require even more land area than larger ones.

SUMMARY AND CONCLUSIONS

Yield records used in these studies were taken from four plantings of sweetpotatoes of the Porto Rico variety, grown in 1929, 1930, and 1931. Two lots were grown near Beltsville, Md., and two at Florence, S.C.

The field data for each planting were assembled in both single and multiple-row plots consisting of one, two, three, four, five, six, eight, ten, and twelve 15-foot row units.

The influence of plot size, shape, orientation, and number of replications on the experimental error in field experiments was determined.

Increasing the size of individual units gave a significant reduction in standard deviation of plots up to about 90 linear feet of row. Increasing the area to more than 90 linear feet of row gave an insignificant reduction in variation between plots as indicated by the standard deviations. Multiple-row plots were less variable than single-row plots of the same total row length and area.

Shape-of-plot studies were made within the limits of the maximum plot size found to give significant reduction in variation, that is, within the limits of 90 linear feet of row. Four shapes of plot were considered, consisting of areas approximately 18 by 15 feet containing 6 parallel rows 15 feet long, 9 by 30 feet containing 3 parallel rows 30 feet long, 6 by 45 feet containing 2 parallel rows 45 feet long, and 3 by 90 feet containing 1 row 90 feet long. With the exception of the 18- by 15-foot plot, the greater dimension paralleled the direction of the rows The data as recorded in 15-foot row units were not adapted to the arrangement of long narrow plots running across the direction of the rows.

Variation among plots decreased as the plot shape approached a square. The 18- by 15-foot areas gave a very significant reduction in variation as compared with the single-row plots 3 by 90 feet.

The study of shape indicates the influence of plot orientation on variation. Variation decreased as the plot dimension at right angles to the direction of the rows was increased. Long narrow plots running across the direction of the rows probably would have given still less variation than was obtained with the plot arrangements used.

The number of replications of the various-sized plots required to obtain significance of a stated percentage difference varied with the different lots of material. Although small plots required a much greater number of replications to secure a given degree of accuracy than did large plots, there was a significant saving in land when small units were employed. The use of small areas has the disadvantage of greater expense and labor in planting and harvesting.

It does not seem advisable to suggest that any one plot size would be the most desirable in sweetpotato field experiments. It is obvious that equally satisfactory results may be obtained from plots of different sizes provided they are replicated a sufficient number of times to meet the statistical requirements. Under the conditions of a specific experiment circumstances might arise to modify the plot technic that otherwise would be the most satisfactory.

A plot consisting of approximately 90 linear feet of row replicated 4 or 5 times should give a satisfactory plot arrangement for field experiments with sweetpotatoes. The data presented indicate that a more significant reduction in variation in plot yields may be expected if the 90 feet of row is divided into a number of shorter parallel rows than where a 90-foot single-row unit is used.

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UNIFORMITY IN PATHOGENICITY AND CULTURAL BEHAVIOR AMONG STRAINS OF THE CABBAGE-YELLOWS ORGANISM[1]

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INTRODUCTION

Yellows is a disease of cabbage and of other members of the species *Brassica oleracea* L., including cauliflower, collards, brussels sprouts, kohlrabi, and kale. The causal organism (*Fusarium conglutinans* Wr.) once established in the soil remains there indefinitely as a potential parasite of these crops. Invasion of the host plant occurs in young roots, and the organism progresses upward by way of the xylem. The characteristic pathological effects have already been described (*4*).[3] As in the case of most of the diseases of herbaceous plants caused by vascular Fusaria, there is in cabbage and its allies marked variation between individuals in resistance or susceptibility to this parasite. This fact has become the basis of control through the selection of resistant strains or varieties (*2*, *3*, *5*). The early work in this direction was done by the selection of survivors from infested fields and by mass selection from standard varieties. More recent work has shown that resistance is controlled by a single dominant Mendelian factor (*1*, *4*), and the discovery of this fact was followed by selection of homozygous resistant lines from a number of varieties of cabbage.

In the program of breeding for disease resistance little attention had been given, up to the time of this investigation, to the possibility of variation in the parasite. Other workers have noted variations in the pathogenicity of vascular parasites of the genus *Fusarium* that attack other plants. It thus became highly important to the program of selection and breeding for resistance to yellows in cabbage and related subspecies that a study of the causal organism be made from the standpoint of its possible variability in pathogenicity. This paper gives the results of such a study carried on from 1927 to the present.

SCOPE OF THE INVESTIGATION

The development of cabbage strains resistant to *Fusarium conglutinans* has been carried on largely in one infested area of Wisconsin. Since specialization occurs not uncommonly among other fungi and has been reported in other vascular parasites of the genus *Fusarium*, the question of specialization was obviously of importance in breeding

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[3] Reference is made by number (italic) to Literature Cited, p. 409.

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cabbage for resistance. Up to the time of this investigation little attention had been given to the question in other than a preliminary way.

The possibility of specialized races of the cabbage-yellows organism may be studied in two ways. First, lines of selected material may be grown upon infested soil in various regions and thus variations in the selective pathogenicity of the parasite may be detected. Such trials have been made in a limited way in the past, and the results have indicated that resistant selections of a number of varieties of cabbage remain resistant wherever tested. Obviously, the results of such experiments are not conclusive because of the possibility of poor infestation of the soil by the pathogene or of environmental factors that might limit full expression of the disease. A second method of determining the existence of specialized races of the pathogene is to study the organism under controlled conditions, whereby various isolates may be compared under optimum conditions that can be provided at will. The results of a study by this method are discussed herein.

METHODS AND MATERIALS

Isolations of the cabbage-yellows organism were secured from various areas of the United States where the disease occurred on cabbage or other subspecies of *Brassica oleracea* (table 1). The material was received as transfer cultures, as diseased plants, or as infested soil. In the last instance cabbage seedlings were transplanted into the infested soil and the yellows organism was isolated from the resultant diseased plants. After the recovery of the organism, monoconidial or hyphal-tip lines were made of isolations 1 to 11. Isolations 12 to 19 were not handled in this way, but were carried in culture as mass transfers from tissue plantings on agar plates. One or more hyphal-tip lines were secured subsequently from each of collections 12 to 19 and were tested for pathogenicity.

TABLE 1.—*Source of isolates of Fusarium conglutinans*

Isolation no.	Locality in which found	Type of material	Date received
1	Racine, Wis	Infested soil	Apr. 1927
2	Ames, Iowa	do	May 1927
3	Crystal Springs, Miss	Diseased collards plant	July 1927
4	Topeka, Kans	Diseased cabbage plant	Do.
5	Houston, Miss	do	Do.
6	La Fayette, Ind	do	Do.
7	Madison, Wis	Diseased kale plant	Do.
8	Indianapolis, Ind	Diseased cabbage plant	Aug. 1927
9	Fayetteville, Ark	Culture isolated from cabbage	Oct. 1927
10	Petaluma, Calif	Culture isolated from kale	May 1928
11	Humboldt, Tenn	Diseased cabbage plant	June 1928
12	Norfolk, Va	do	June 1930
13	Lansing, Ill	do	July 1930
14	Fremont, Ohio	do	Do.
15	Columbus, Ohio	do	Do.
16	Somers, Wis	do	Do.
17	Indianapolis, Ind	do	Do.
18	Nekoosa, Wis	do	July 1931
19	Madison, Wis	do	Aug. 1931

The comparative pathogenicity trials of the isolates were conducted by transplanting cabbage seedlings (or those of other subspecies of *Brassica oleracea*) into soil that had been inoculated with cornmeal-sand cultures of the several isolates. The cornmeal-sand medium was prepared by mixing equal parts of yellow cornmeal and white quartz sand, moistening with water, and steaming for 1 hour. Approximately equal parts of the mixture were then placed in quart fruit jars, plugged with cotton, and autoclaved on 3 alternate days. Tube cultures of the isolates were used to inoculate the jars of the cornmeal-sand medium, which were incubated at room temperature for a period of 2 weeks or more. The inoculum was then mixed thoroughly with clean compost soil and placed in the metal cans of the Wisconsin soil-temperature tanks. Seedlings that had been grown in yellows-free soil for a period of 20 days or more were transplanted into the cans of artificially inoculated soil and exposed to the attack of the isolates under conditions of controlled soil temperature maintained at 22° to 24° C.

In these comparative pathogenicity studies identical conditions of inoculum and uniform conditions of soil moisture and soil temperature were maintained as closely as possible. Care was exercised at all times to eliminate the possibility of contamination of one isolate by another during the process of soil inoculation, transplanting, etc.

For the pathogenicity trials a number of varieties and strains of cabbage and other subspecies of *Brassica oleracea* were used. Homozygous susceptible lines of cabbage developed by Walker (*4*) were used, originating from several commercial varieties, as follows:

(1) HC-27-A, from a cross between All Head Early and Copenhagen Market varieties.
(2) C 29-A, from Copenhagen Market variety.
(3) SP-GC, from Late Flat Dutch variety.

Homozygous resistant lines of cabbage also developed by Walker (*4*) were used. These had been shown to remain completely resistant on infested soil up to about 24° C. Above 26° they developed disease symptoms not typical of yellows, which are discussed in detail by Walker and Smith (*6*). The following lines were used:

(1) 20-28-A and 20-30-B, developed from Jersey Wakefield variety.
(2) 30-28-A, developed from Copenhagen Market variety.
(3) 40-28-A, developed from All Head Early variety.

A number of F_2 progenies from crosses between resistant and susceptible strains of cabbage used by Walker were included. Under Wisconsin field conditions he found these to segregate close to the ratio of 3 resistant to 1 susceptible. The progenies, exact pedigrees of which are given by Walker (*4*), were as follows: 5HIA, H5ID, G201-1s, G51C.

In the comparative study of subspecies of *Brassica oleracea* commercial lots of seed were used, since selected lines were not available.

EXPERIMENTAL RESULTS

TRIALS WITH HOMOZYGOUS LINES OF CABBAGE

The initial pathogenicity trials of the yellows cultures were made upon seedlings of a commercial susceptible variety of cabbage. After typical symptoms of the yellows disease had been produced, the causal organism was isolated and new lots of the cornmeal-sand medium were inoculated with the cultures of proved pathogenicity. The isolates bearing accession numbers 1 to 11, inclusive, were purified by obtaining monoconidial or single hyphal-tip lines; isolates

12 to 19, inclusive, were not so handled in the early trials, although later single hyphal-tip lines were obtained and studied.

The first comparative trial of cultures 1 to 11 was made upon cabbage seedlings of 2 homozygous susceptible and 2 homozygous resistant lines. After 17 days' exposure in infested soil at a soil temperature of 24° C. the seedlings of the homozygous susceptible lines were all diseased, whereas the seedlings of the resistant lines showed no evidence of the disease. Although there was no indication of wide variation among the isolates, the number of seedlings used in the trial was too small to give great significance to the results.

In the next trial the number of seedlings was increased so that any variation in virulence might be more readily detected. The results presented in table 2 show the reaction of 1 homozygous susceptible line (HC-27-A) and 3 homozygous resistant lines (20-28-A, 30-28-A, and 40-28-A) to 10 isolates. All plants of the susceptible line succumbed to each isolate except no. 6, which induced disease in 93 percent of the seedlings. The lower pathogenicity in this case was explained by a later demonstration that a nonpathogenic contaminant was present in the inoculum jar, resulting in a dilution of the pathogene and consequently in a less virulent attack. The three resistant lines of cabbage remained entirely free from disease symptoms. The data presented in table 2 are typical of other trials, not presented here, with similar seedling material and the same cultures of the fungus.

Table 2.—*Pathogenicity of isolates of Fusarium conglutinans upon 3 homozygous resistant strains and 1 homozygous susceptible strain of cabbage*

Isolate no.	Plants of homozygous susceptible strain— HC-27-A		Plants of homozygous resistant strain—					
			20-28-A		30-28-A		40-28-A	
	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	60	100	80	0	45	0	79	0
2	60	100	79	0	44	0	79	0
3			83	0			84	0
4	59	100	80	0	48	0	80	0
5	62	100	82	0	44	0	80	0
6	61	93	81	0	44	0	81	0
8	64	100	67	0	44	0	80	0
9	60	100	79	0	45	0	75	0
10	60	100			47	0		
11	62	100	80	0	45	0	77	0

The comparative pathogenicity trials were next extended to isolates 12 to 19, inclusive. In table 3 are presented the results of trials upon homozygous susceptible and homozygous resistant lines of cabbage. In three successive trials with a susceptible line (C-29-A) pathogenicity was uniform except in the case of isolate 16, which was less virulent than the others. Upon a second susceptible line (SP-GC) this strain eventually brought about disease symptoms in all of the seedlings, but the disease progressed much more slowly than in the case of the other isolates. The resistant lines gave no evidence of disease.

TABLE 3.—*Pathogenicity of isolates of Fusarium conglutinans upon 2 homozygous resistant and 2 homozygous susceptible strains of cabbage*

Isolate no.	Plants of homozygous susceptible strain—								Plants of homozygous resistant strain —			
	C-29-A						SP-GC		20-30 B		40-28-A	
	Trial 1		Trial 2		Trial 3							
	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
12	45	100	10	90	8	88	15	100	55	0	60	0
13	45	100	10	100	8	100	15	100	73	0	75	0
14	45	98	10	100	8	100	15	100	75	0	75	0
15	45	100	10	100	8	100	15	100	74	0	60	0
16	45	42	10	80	8	25	15	100	75	0	75	0
17	45	100	10	100	8	88	15	100	75	0	75	0
18	45	98	10	100	8	88	15	100	57	0	60	0
19	45	100	10	100	8	88	15	100	70	0	75	0

Single hyphal-tip lines were secured from isolates 12 to 19. Three lines were secured from isolate 15; two lines each from isolates 13, 16, 17, and 19; and one line each from isolates 12, 14, and 18. They were increased on cornmeal-sand medium and introduced into clean soil. Two homozygous susceptible lines and one homozygous resistant line of cabbage were tested. The results, given in table 4, again show no evidence of infection of the resistant line. The data in table 3 show reduced virulence in the original isolate 16 but not in isolate 15. It is apparent, however, that all the lines derived from these isolates 15 and 16 showed reduced virulence upon the susceptible strains of cabbage. This is the only evidence obtained in this study of any marked variation in virulence. It is to be noted, however, that it was variation in the direction of lower virulence on susceptible cabbage and not in the direction of increased virulence upon resistant cabbage.

TABLE 4.—*Pathogenicity of hyphal-tip lines from isolates of Fusarium conglutinans upon homozygous susceptible and homozygous resistant strains of cabbage*

Hyphal-tip line	Plants of homozygous susceptible strain —				Plants of homozygous resistant strain 40-28-A			
	C-29-A		SP-GC		Trial 1		Trial 2	
	Total	Diseased	Total	Diseased	Total	Diseased	Total	Diseased
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
12-1	12	100	13	100	12	0	10	0
13-1	12	92	13	100	12	0	10	0
13-2	12	92					10	0
14-1	12	100	13	100	12	0	10	0
15-1	12	83	13	46	12	0	10	0
15-2	12	58					10	0
15-3	12	50					10	0
16-1	12	8	13	23	12	0	10	0
16-2	12	25					10	0
17-1	12	100	13	100	12	0	10	0
17-2	12	100					10	0
18-1	12	92	13	92	12	0	10	0
19-1	12	100	13	100	12	0	10	0
19-2	12	100					10	0

TRIALS WITH F_2 HYBRIDS FROM CROSSES BETWEEN RESISTANT AND SUSCEPTIBLE LINES OF CABBAGE

The inheritance of resistance in cabbage to the yellows organism, *Fusarium conglutinans*, has been found to be based upon a single dominant factor (*4*). F_2 hybrids of crosses between resistant and susceptible plants segregate in the second generation in the ratio of 3 resistant to 1 susceptible. The availability of seed led to a study of the reaction of 4 F_2 progenies to 9 of the most virulent isolates of the fungus. It is to be seen from the results in table 5 that all progenies segregated very closely in the expected ratio of 3 resistant to 1 susceptible when exposed to each isolation of the yellows organism.

TABLE 5.—*Pathogenicity of 9 isolates of Fusarium conglutinans upon F_2 hybrids from resistant susceptible crosses*

Isolate no.	F_2 strain											
	5HIA			H5ID			C201-1s			G5IC		
	Healthy plants	Diseased plants	Deviation, probable error [a]	Healthy plants	Diseased plants	Deviation, probable error	Healthy plants	Diseased plants	Deviation, probable error	Healthy plants	Diseased plants	Deviation, probable error
	Number	*Number*		*Number*	*Number*		*Number*	*Number*		*Number*	*Number*	
1	46	9	2.2	43	15	0.2	38	14	0.5	12	4	0
2	42	16	.7	48	14	.7	38	14	.5	13	3	
4	42	15	.3	47	10	1.9	39	13	0	20	10	1.
5	46	15	.1	49	11	1.8	40	12	5	21	9	.
6	40	19	1.9	40	19	1.9	40	12	.5	23	7	.
8	46	15	.1	45	15	0	41	11	.9	22	8	.
9	46	12	1.1	44	16	.4	37	15	.9	12	4	0
10	46	15	.1	41	19	1.8	39	13	0	23	7	.
11	44	17	.8	43	16	.6	39	13	0	25	5	1

[a] Deviation from the expected segregation of a monohybrid, i. e., 3 healthy to 1 diseased.

TRIALS WITH CERTAIN SUBSPECIES OF BRASSICA OLERACEA

Eight of the isolates were tested upon New Snowball cauliflower, White Vienna kohlrabi, Moss-Curled and Thousand-Headed kale, and Copenhagen Market cabbage. All these were commercial lots of seed and were therefore not expected to be uniform for resistance or susceptibility. Walker and Wellman (*7*) in field trials showed these subspecies to contain varying percentages of resistant individuals when commercial lots were used. The results of this trial (table 6) show that each subspecies contained a high percentage of individuals susceptible to each isolate. There are some fairly wide variations in the percentage of individuals of a given variety infected by the various isolates. This may be accounted for by the fact that the population of a given variety was homozygous neither for resistance nor susceptibility, and therefore a much larger number of test plants in each case would be necessary to establish the significance of such differences. It is clear, however, that all varieties of the subspecies used were relatively highly susceptible to the isolates used. Similar trials with Long Island Improved brussels sprouts and Georgia collards gave corresponding results. In none of these tests was there any evidence of selective pathogenicity for one or more of the subspecies of *Brassica oleracea.*

TABLE 6.—*Pathogenicity of 8 isolates of Fusarium conglutinans upon subspecies of Brassica oleracea* [a]

Isolate no.	Diseased plants in—				
	Cabbage	Cauliflower	Kohlrabi	Moss-Curled kale	Thousand-Headed kale
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
	90	67	57	85	62
	92	85	64	92	58
	100	100	71	100	80
	100	77	69	92	52
	85	75	67	67	85
	85	75	67	77	50
	100	100	71	94	59
	92	58	53	92	61

[a] The number of plants in each test of each isolate ranged from 10 to 23.

CULTURAL STUDIES

Throughout the routine transfer of the cultures of the fungus and the frequent reisolations from diseased plants it was noted that the isolates resembled one another closely in gross cultural characteristics. Certain experiments were conducted to compare more accurately their cultural characters. The temperature range over which the cultures were observed was from 20° to 28° C. The isolates were compared as to rate of colony growth, spore production, and pigment formation. The media used were malt-extract agar, potato-dextrose agar, steamed rice, oatmeal agar, and Richards' nutrient solution.

The rate of growth of the fungus, as measured by increase in colony diameter, was studied on potato-dextrose and malt agars in Petri dishes. Precautions were taken to insure equal conditions for the comparative studies, and the plates of a single isolate were made in triplicate. Isolates 1 to 11 were studied as one group; isolates 12 to 19 and the hyphal-tip lines derived from them were compared later. The growth rates of all fell within fairly narrow limits. The optimum temperature for all appeared to be that commonly reported for *Fusarium conglutinans*, namely, about 25° to 27° C. All displayed similar gross characteristics as to character and amount of aerial mycelium. It was noted, however, that the colony margin of isolate 10 was more sharply defined than that of the others, and that the aerial growth of isolate 16 was somewhat less abundant than the aerial growth of the others. Although the cultural characters of isolate 15 were typical of those commonly described for *F. conglutinans*, the hyphal-tip lines derived from it displayed considerable variation in the character and amount of aerial growth. The cultures were compared as to color production upon the above-mentioned agars and upon oatmeal agar, steamed rice, and Richards' nutrient solution. None produced color upon any of the media except steamed rice, where a salmon-pink color was produced by all. Microconidia and chlamydospores were produced by all, whereas macroconidia were found only occasionally. Saltations were not uncommon in the hyphal-tip lines of isolate 15, whereas the other collections were apparently very stable and failed to show this type of variation.

DISCUSSION

Cultural studies of a number of isolations of the cabbage-yellows organism failed to bring out significant differences in their behavior. These studies included comparisons as to rate of growth, color production, and sporulation. It is not the writer's contention that the isolates of the fungus that were studied are identical, since certain minor differences in their behavior were evident. The variations that appeared during the cultural work are being studied further to determine whether or not they are of significance. During the routine handling and observation of the cultures there has been evidence of sectoring only in the hyphal-tip lines of one of the isolates. In general the numerous isolates of the organism have been remarkably uniform in their behavior.

The data accumulated in the pathogenicity trials upon selected lines of cabbage and upon other subspecies of *Brassica oleracea* are in accord with the general results of the cultural studies. The isolates gave one general type of reaction in which all concur. Lines of cabbage selected for susceptibility were uniformly attacked by all except isolate 16 and the hyphal-tip lines from isolate 15, which differ only in showing a reduced degree of virulence toward susceptible plants.

Further evidence of the uniformity of the isolates is presented in the comparative pathogenicity trials upon the F_2 hybrids, in all of which a close fit to the expected 25 percent of diseased plants was obtained. No selective pathogenicity was displayed in trials with other subspecies of *B. oleracea.* Selected lines of cabbage that were homozygous for resistance showed no symptoms of the yellows disease when exposed to each isolate at a soil temperature of 24° C.

Although strains of *Fusarium conglutinans* may occur which do not conform in their pathogenic properties to those studied, the wide geographic range covered by this collection of cultures strongly indicates that this species is very stable in its selective pathogenicity. There is reason to believe, therefore, that the varieties of the various subspecies of *Brassica oleracea* selected for resistance in one locality will remain stable for this character in other localities.

SUMMARY

A study was made of the comparative pathogenicity and cultural behavior of 19 isolates of the causal organism of cabbage yellows, *Fusarium conglutinans* Wr., from 11 States.

Comparative cultural studies of the 19 isolates and hyphal-tip lines derived from certain of them were made as to growth rate, spore production, and color production. The growth rates of all were similar when tested over a series of temperatures, no outstanding differences being noted. Microconidia and chlamydospores were produced by all isolates; macroconidia were not consistently present. On steamed rice all isolates produced a salmon-pink to flesh color after a week or more in strong diffused light. Sectoring was present only in the hyphal-tip lines of one isolate.

Homozygous susceptible lines of cabbage were uniformly attacked by all except two of the isolates, in which the organisms were less virulent upon homozygous susceptible cabbage.

All isolates of the fungus were alike in their inability to attack successfully the homozygous resistant lines of cabbage at 24° C.

In the F_2 hybrid lines of resistant-susceptible crosses the percentage of plants that became diseased when tested against nine isolates was in all cases close to the expected 25 percent.

The isolates showed no evidence of selective pathogenicity upon the six subspecies of *Brassica oleracea* that were studied.

From the results presented in the foregoing data it is safe to assume that specialization is not a vital factor in the program of selection and breeding for resistance to the cabbage-yellows pathogene.

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TOXIN PRODUCED BY BACTERIUM TABACUM AND ITS RELATION TO HOST RANGE[1]

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INTRODUCTION

Since 1918, when the wildfire disease of tobacco (*Nicotiana tabacum* L.) was originally described by Wolf and Foster (*13*),[3] reports of its host range have varied from tobacco only to a large number of species in many genera. Wolf (*12*) and Clinton and McCormick (*3*) considered the causal organism, *Bacterium tabacum*,[4] as limited to tobacco species. Chapman and Anderson (*2*), however, reported infection on eggplant, pokeweed, and petunia, and observed naturally occurring lesions on tomato. In 1924 Johnson, Slagg, and Murwin (*8, p. 180*) reported results from inoculation experiments in which they secured infection on species of 23 genera with indications that "the host range of *Bact. tabacum* is very much larger than indicated by these results." In 1929 Săvulescu and Radulescu (*11*) reported a study of a serious tobacco disease in Rumania which they ascribed to *Bact. melleum* Johnson, but which appears from their publication to be much more like wildfire. A feature of their report was a very extensive host range, with conclusions as to susceptibility of various families and species, which correspond closely with those reported by Johnson, Slagg, and Murwin (*8*).

A complicating factor, however, was introduced by the discovery of Johnson and Murwin (*7*) in 1925 that *Bacterium tabacum* secretes a toxic substance in culture, and that this substance when filtered free from bacteria and inoculated into leaves produces typical symptoms of the wildfire disease. Johnson and Murwin point out that this might raise a question as to the previously described host range, but conclude that "in most cases at least the organism was actively parasitic, although the initial symptoms may have been produced by toxin introduced from cultures."

The present study was undertaken as part of a general investigation of the tobacco leaf-spot diseases.

TOXIN PRODUCED BY BACTERIUM TABACUM IN PURE CULTURE

Bacterium tabacum grows well on a variety of media, both liquid and solid, and produces toxin freely on all media tested. The usual potato-dextrose broth proved well adapted for the experimental work, and was used throughout the tests. As previously indicated, the toxin can be secured free from bacteria by filtration, and the same

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[2] The writer is greatly obligated to Dr. James Johnson, of the University of Wisconsin, for many helpful suggestions.

[3] Reference is made by number (italic) to Literature Cited, p 425.

[4] Synonym: *Phytomonas tabaca* (Wolf and Foster) Bergey et al.

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result can be secured by heat or chemical sterilization. It has been possible to demonstrate by these different methods that as early as 24 hours after transfer toxin in appreciable amounts is present in culture tubes incubated at 20° to 25° C. Cultures 48 hours old contain sufficient toxin to produce large leaf lesions, and cultures 5 to 7 days old may be diluted with water up to 16 times and still give a strong positive reaction when inoculated into the leaf. The accumulation of toxin in liquid culture gradually ceases, and tests with cultures 6 months old have shown no toxin, although living bacteria were still present. That the bacteria in these cultures had actually destroyed their own toxin was proved by the fact that similar cultures sterilized when 2 weeks old contained an abundance of toxin at the end of the 6 months' storage.

PROPERTIES OF TOXIN

The fact that cultures can be freed from the bacteria by passage through a Berkefeld filter and that the toxin is present in the filtrate has been established by Johnson and Murwin (7). This work was repeated and the results confirmed. In addition, collodion, collodion plus 4 percent glycerin, and cellulose sacks were prepared. These three types of sacks were tested by filling with water, and all showing any signs of defect were rejected. The selected sacks were then filled with (1) a sterile Berkefeld filtrate and (2) 3-day-old *Bacterium tabacum* cultures. The filtrate secured from the sacks was inoculated into leaves, and in each case the presence of abundant toxin was demonstrated. Agar dilution plates were poured from the culture filtrate (2) and also from inoculated plants and no *Bact. tabacum* bacteria were secured. These results indicated that the toxin was actually excreted by the bacteria, that it could be secured without destroying the bacteria, and that the toxin particles were very small.

Since the toxins known to animal pathology and also the virus causing mosaic disease of tobacco can be precipitated readily by lead acetate, the action of this substance and that of calcium acetate on the *Bacterium tabacum* toxin was tested. The procedure followed with neutral lead and calcium acetates was to add an excess of a 5-percent solution to separate portions of the filtrate from a 12-day-old broth culture. This treatment raised the pH value from an original 4.3 to about 5. As there was no precipitate in the portion treated with calcium acetate, this portion was held without further treatment. The portion treated with lead acetate showed a thick gellike precipitate which was removed by centrifuging, and the clear liquid retained. The excess of lead was precipitated with sulphuric acid and removed, and the clear liquid was brought to a pH of 6.6 with potassium hydroxide. Leaf-prick inoculations were made with (1) the original culture-solution filtrate, (2) the portion treated with calcium acetate, (3) the clear liquid left after treating a portion of one with lead acetate, and (4) the same after precipitating the excess of lead and restoring to a pH of 6.6. The inoculation results were promptly and strongly positive with each lot, indicating that none of the treatments had affected the toxin.

Diluting a culture solution with an equal volume of absolute alcohol did not precipitate or destroy the toxin. The alcohol mixture gave positive results on inoculation, as did also the clear solution remaining after the alcohol had been driven off by heat, and the precipitate removed. After a culture solution had been boiled with bone black and filtered, the toxin was still present in the filtrate.

Sulphuric and hydrochloric acids and sodium and potassium hydroxides were used to test the action of acids and alkalies. The toxin was completely and rapidly inactivated by 0.25 percent of sodium or potassium hydroxide, and furthermore the inoculated leaves developed no infections even after a month or more, indicating destruction of both toxin and bacteria. Ammonium hydroxide of similar strength, however, did not affect the toxin. Potassium carbonate, a basic salt, destroyed the toxin, though less rapidly than did the hydroxide, and a stronger solution was required. The action of alkalies on the toxin was not instantaneous, but took place over a considerable period as indicated in table 1. The action of the alkalies was not reversible, since solutions of toxin inactivated by treatment with sodium hydroxide and then restored to their original pH by adding hydrochloric acid continued to give negative results. Neither hydrochloric nor sulphuric acid of strength up to 0.5 percent inactivated the toxin.

TABLE 1.—*Results from inoculations with toxin solutions to which various chemicals had been added*

[15 inoculations were made for each test]

Chemical added to toxin solution	Strength of chemical	Inoculations made— 1 hour after treatment: Negative	Inoculations made— 1 hour after treatment: Positive	Inoculations made— 24 hours after treatment: Negative	Inoculations made— 24 hours after treatment: Positive
	Percent	*Number*	*Number*	*Number*	*Number*
KOH	0.10	11	4	15	0
	.15	12	3	15	0
	.20	15	0	15	0
	.25	15	0	15	0
	.30	15	0	15	0
NaOH	.25	15	0	15	0
K_2CO_3	.25	0	15	0	[a] 15
	.50	9	6	15	0
	1.00	9	6	15	0
Untreated	--	0	15	0	15

[a] Very faint

It is to be noted that the weaker strengths of the hydroxide (0.1 and 0.15 percent) were completely effective in destroying the toxin after 24 hours and only partly effective after 1 hour. The value of the longer treatment was also evident with the potassium carbonate, which in strengths of 0.5 and 1 percent was completely effective after 24 hours and only partly effective after 1 hour. These and similar results would seem to indicate a slow breaking down of the toxin by the alkali rather than an instantaneous reaction.

To test the effect of a powerful disinfectant, 10-day-old cultures were treated so as to give 0.0375, 0.075, 0.15, and 0.3 percent solutions of mercuric chloride. After 12 hours tobacco leaves were inoculated with these solutions. Transfers were made from each treated lot to tubes of sterile broth, to check for the presence of living bacteria. The leaf inoculations gave strong positive results, indicating an abundance of toxin in all. The transfers showed that even the lowest mercuric chloride concentration had destroyed all bacteria. Similar tests were conducted with formaldehyde, and here again the bacteria were readily destroyed by comparatively low concentrations, but the toxin was not affected.

It was early found that this toxin had very high thermal stability. Originally temperatures of 50° to 60° C. were used, with exposures of 10 to 60 minutes, the plan being to find an exposure that would destroy the bacteria but not injure the toxin. On investigation, however, it was discovered that even steaming in the autoclave for 30 minutes with 15 pounds pressure did not inactivate the toxin, nor did boiling over a free flame for 3 hours, during the course of which the volume of the solution was reduced 87 percent. It was shown, however, by testing the rediluted solution, that this prolonged boiling had destroyed a part of the toxin.

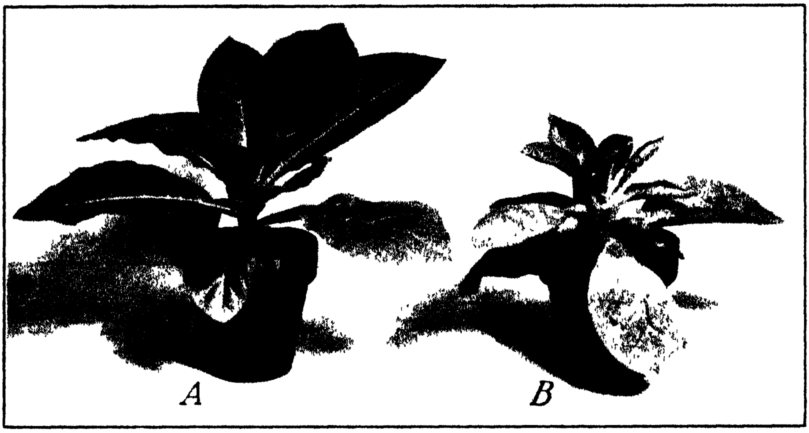

FIGURE 1.—Tobacco plants, showing effect of toxin of *Bacterium tabacum* on entire plant after inoculation of vascular tissues of stem: *A*, Uninoculated plant used as check; *B*, plant 6 weeks after inoculation of stem; the entire plant was yellowed and stunted, although the bacteria were present only in a small area around the point of inoculation.

ACTION OF TOXIN ON PLANT TISSUES

The wildfire leaf lesion is very characteristic in appearance, so much so that the organism is usually identified by inoculating leaves and watching for the development of the large yellow halo spots. Their circular shape indicates a diffusion of some substance out from the central point of infection, and investigation showed that the color of the halo was due to destruction of the green chlorophyll, which permitted the yellow carotene pigment to become visible. The chlorophyll of many plants was so sensitive to this toxin that a strong solution applied to uninjured leaves caused the treated areas to turn a yellow green. Additional proof of the very powerful action of the toxin on chlorophyll was supplied by inoculating greenhouse tobacco plants in the stem (fig. 1). The leaves above the inoculation turned yellow, though the actual infection usually remained confined to a small area near the point of inoculation. Indications of a definite chemical reaction between the toxin and chlorophyll were supplied by experiments in which a fresh alcohol extraction of chlorophyll and a culture of *Bacterium tabacum* were mixed half and half. After several days a distinct precipitate was formed which on settling out left a yellow-green liquid in place of the original green. Similar tests with an organism such as *Bact. angulatum* Fromme and Murray, which secretes no such toxin, gave no reaction.

Further evidence of a specific action of the toxin on chlorophyll was supplied by the following experiment: Lots of tobacco and bean-leaf tissue were extracted with alcohol overnight, and then liquid cultures of *Bacterium tabacum* were treated with equal portions of (1) pure alcohol and (2) the alcoholic chlorophyll extracts. After standing 5 days, the alcohol was evaporated by heating and leaves were prick inoculated. The halos obtained with 1, the alcohol-treated portion, were very large and clear; but with 2, the alcohol-chlorophyll-treated lots, only negative to faintly positive results were secured, indicating that the toxin had been largely inactivated.

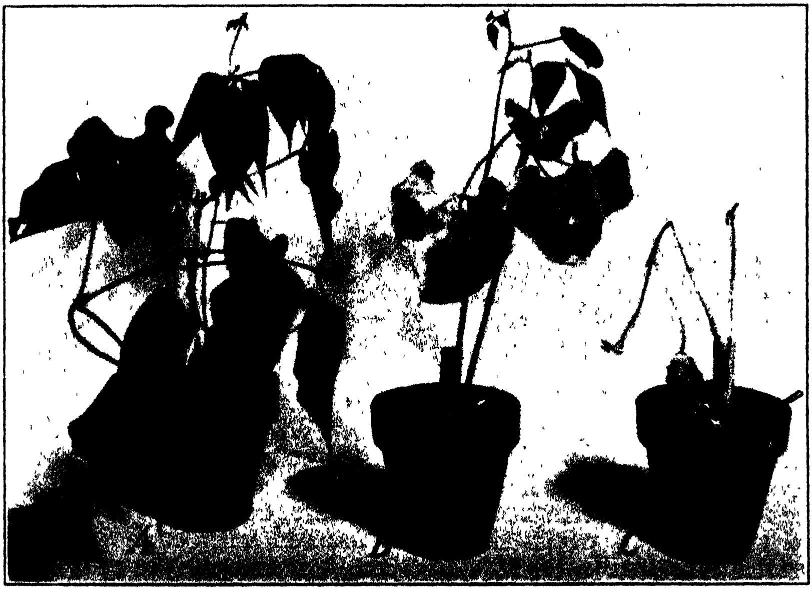

FIGURE 2.—String bean plants showing extreme susceptibility to the toxin of *Bacterium tabacum*. *A*, Check plants sprayed with water. *B*, Plants sprayed with ordinary liquid culture of *Bact. tabacum* diluted with an equal volume of water, plants then held at 55° to 65° F. *C*, Plants treated as in *B* but held at 70° to 80°. Similar results were secured by means of sterile cultures. Tobacco plants treated in same manner showed a slight yellowing of some leaves but suffered no permanent injury from the toxin.

The chlorophyll of many kinds of plants is attacked by this toxin, and wide variations in susceptibility exist. Tobacco is only moderately susceptible, whereas the string bean (*Phaseolus vulgaris* L.) is extremely susceptible. Thus bean plants may be completely killed by spraying with a toxin solution that only slightly injures tobacco plants (fig. 2). The action of the toxin is subject to the usual temperature laws, being greatest at high temperatures.

GREENHOUSE EXPERIMENTS

INOCULATIONS

Owing to the difficulty of properly controlling conditions in the field, most inoculation experiments are conducted in the greenhouse, and this has been true of the previous work with *Bacterium tabacum*.

The writer's experiments have covered a period of 2 years, and large numbers of tests under a wide variety of conditions have been made. The results secured are summarized, since a presentation of the separate data would require far too much space. The following is a brief statement of the methods employed.

Wound inoculations were made with glass tubes drawn to a point and broken off to give a small opening. The tube was filled with the inoculating liquid, and, as a prick was made, a drop of the fluid was left on the leaf. Spray inoculations were made with the usual atomizers, and in either case the plants were placed in a damp chamber for 3 to 5 days after inoculation. The work was generally conducted at temperatures between 70° and 80° F. Since the usual culture contained both bacteria and toxin, it was thought necessary, in addition to inoculating with such cultures, to make comparative tests with the bacteria and toxin separately. Bacteria free from toxin were secured by centrifuging cultures, pouring off the supernatant liquid, resuspending the precipitate in distilled water, and repeating this procedure twice. The suspension of bacteria so obtained gave no test for toxin if heated and inoculated, and showed an abundance of living bacteria as evidenced by microscopic examination and by heavy infection resulting from spray or wound inoculations. Owing to its stability, it was very easy to secure solutions of the toxin free from bacteria by sterilizing with heat and filtering through a Berkefeld, or by treating with 0.05 percent mercuric chloride.

It was soon apparent that short incubation periods of 2 to 3 days obtained with wound inoculations were largely the result of toxin contained in the inoculating fluid. This quick appearance of infection was secured either with the culture solution containing both toxin and bacteria or with the toxin alone. When bacteria alone were used on tobacco leaves in a susceptible condition, usually about 7 days were required before positive results were apparent (fig. 3). The subsequent development of the disease, however, was the same, and after 3 to 4 weeks the lesions secured with bacteria or with bacteria plus toxin were similar. The lesions from the toxin alone, on the other hand, soon ceased to develop, and after a few weeks were less conspicuous than the others, indicating that with tobacco the first symptoms produced by the toxin of the inoculating fluid are supplemented by further symptoms as the result of toxin produced by the bacteria in the leaf tissues.

Inoculations were made on many other plants, including cucumber (*Cucumis sativus* L.), cantaloup (*Cucumis melo* L.), bean (*Phaseolus vulgaris* L.), *Begonia* sp., eggplant (*Solanum melongena* L.), bindweed (*Convolvulus arvensis* L.), *Geranium* sp., lantana (*Lantana camara* L.), oat (*Avena sativa* L.), petunia (*Petunia hybrida* Hort.), pepper (*Capsicum annuum* L.); poinsettia (*Euphorbia pulcherrima* Willd.), tomato (*Lycopersicon esculentum* Mill.), mustard (*Brassica nigra* (L.) Koch), and zinnia (*Zinnia elegans* Jacq.). The results from inoculations with toxin or with toxin plus bacteria were positive. In all of these the lesions appeared promptly and were large and characteristic of the wildfire disease (figs. 4 and 5). In the case of some of these plants the lesions appeared very quickly and were in every way larger and more conspicuous than those secured in the comparative inoculations on tobacco. Positive results of this sort, however, were never secured from inoculations with the bacteria alone. Not infrequently a narrow margin of dead tissue developed around the inoculation wound and, in an extremely toxin-susceptible plant like the bean, small halo lesions occasionally appeared. There was, however, no subsequent development of the lesions, and it is a significant fact that with all plants other than tobacco the lesions, whatever the inoculum used, developed to a maximum size within 10 days, whereas with tobacco they often continued to enlarge over a period of weeks. This difference in behavior is to be associated with information presented later on the ability of the organism to persist and multiply in the tissues of various plants. The main result from the extensive series of wound-inoculation experiments was the fact that whereas tests with toxin or toxin plus bacteria indicated that there were many plants susceptible to the disease and some of them very much more susceptible than tobacco, as judged by the size of lesions secured, tests with the bacteria alone failed to give positive results, except with tobacco.

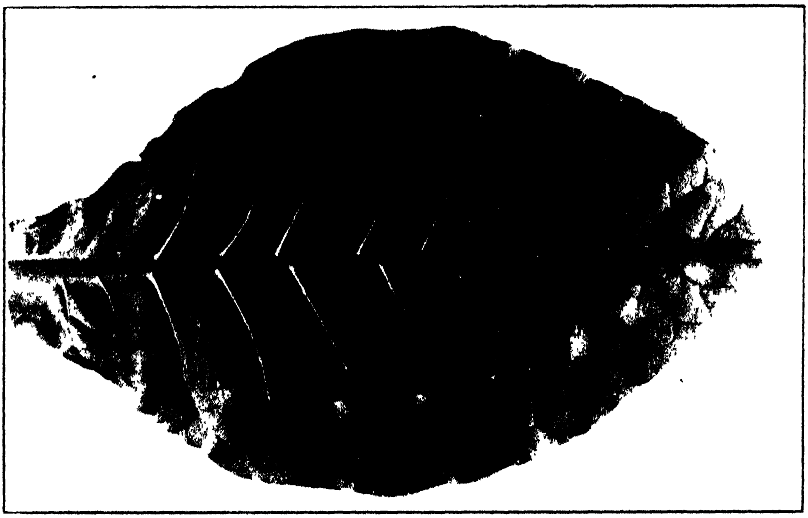

FIGURE 3.—Tobacco leaf inoculated with *Bacterium tabacum*. The lower half was wound-inoculated with an 8-day-old culture containing an abundance of bacteria and toxin; the upper half, with bacteria washed free from toxin. Photograph taken 8 days after inoculation, when halo lesions resulting from bacteria plus toxin ranged in diameter up to three eighths of an inch, with a fringe of dead tissue immediately around the central prick. Inoculations with bacteria alone were just beginning to show a faint yellowing. Three weeks later lesions resulting from bacteria plus toxin and those from bacteria alone were equally well developed.

In addition to the wound inoculations, many tests were made by atomizing the leaves with diluted cultures, or suspensions of bacteria free from toxin. Other investigators have noted that this is a less certain method of obtaining infection than wounding. However, it was found that by providing suitable conditions good infection could be secured on uninjured tobacco leaves. Inoculations on other plants generally gave no infection, but by repeated and extensive tests it was found that occasionally some lesions could be secured. Such lesions were obtained on cantaloup, cucumber, eggplant, and tomato. The difference in the results from spray inoculations on these plants as compared with those on tobacco was in the amount of infection. Thus with conditions so favorable as to give up to 500 infections on individual tobacco leaves, 2 to 5 infections might result on the inoculated leaves of some of the other plants.

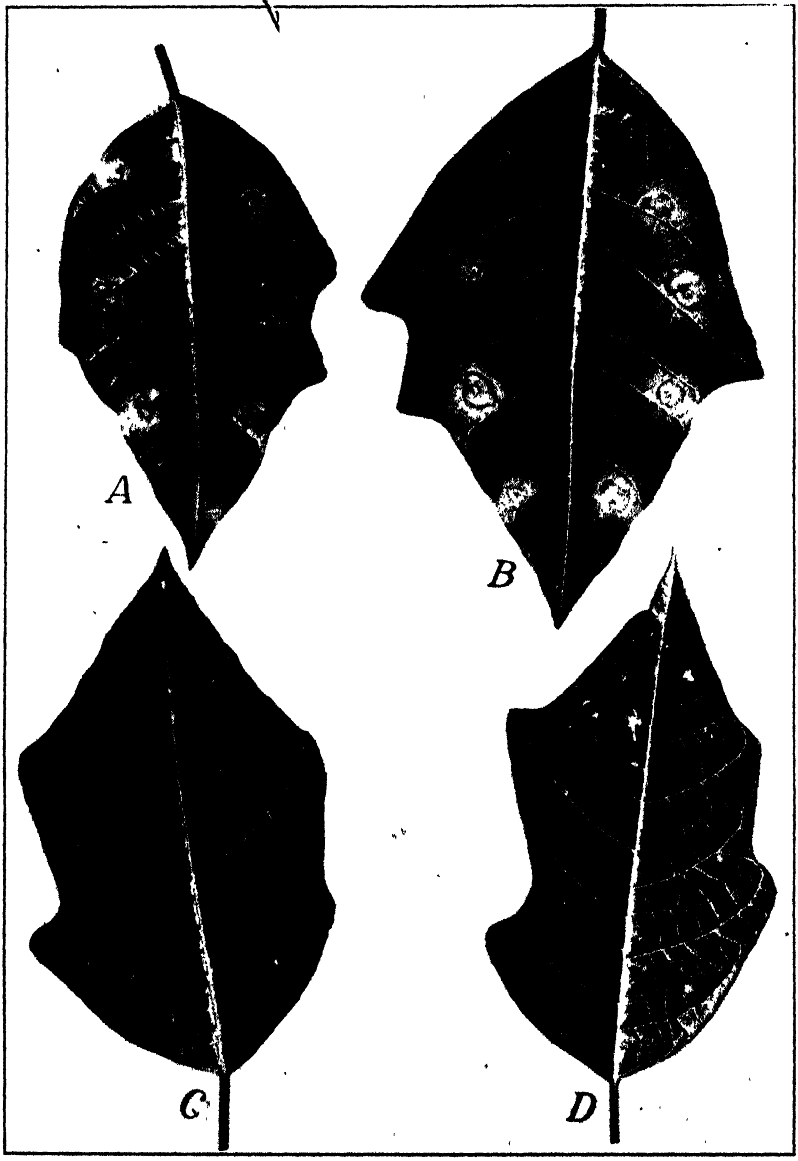

FIGURE 4.—Poinsettia leaves inoculated at same time and in same manner as tobacco leaf (fig. 3). *A* and *B*, Leaves inoculated with bacteria plus toxin; lesions ranged in diameter up to five eighths of an inch; central dead areas ranged in diameter up to three eighths of an inch. *C* and *D*, Leaves inoculated with bacteria free from toxin; some pricks had a narrow fringe of dead tissue, but there was no further development of lesions, even after 6 weeks. Note that the striking results on *A* and *B*, without the check on *C* and *D*, would indicate that the poinsettia is more susceptible than tobacco to *Bacterium tabacum*.

ISOLATIONS

In previous work on host range of *Bacterium tabacum* investigators in some instances have regarded the appearance of the characteristic leaf lesions as positive proof of infection; in other cases they have reisolated the organism and proved its pathogenicity by reinoculation, thus completing the proof as prescribed by Koch. The present writer has found that after wound inoculations it is quite possible to reisolate the organism from many plants, but that the longer this reisolation is delayed the more difficult it becomes, although leaf symptoms may continue prominent. The writer's tests indicated that in plants other than tobacco and susceptible species of *Nicotiana* the bacteria effect a temporary lodgment, but instead of multiplying and spreading they rapidly decline in number. The difference in the number of bacteria present 3 weeks after inoculation in tobacco, as compared with that in other plants, was readily shown by poured plates. The procedure followed was to remove with a small cork borer equal areas from lesions of similar age, wash in sterile water, crush, and pour dilution plates. An example of the data so secured is given in table 2.

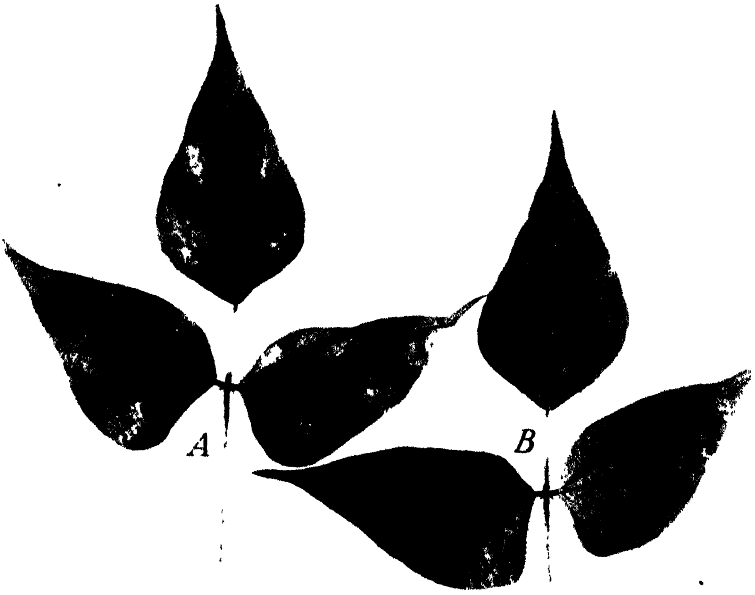

Figure 5.—Bean leaves inoculated at same time as tobacco leaf (fig 3). *A*, Leaves inoculated with toxin, lesions ranged in diameter up to 1⅛ inches; central dead areas ranged in diameter up to one half inch *B*, Leaves inoculated with bacteria; few small lesions, which made no further development. Bean-leaf inoculations with toxin alone frequently gave even larger lesions than did inoculations with toxin plus bacteria.

Table 2.—*Dilution-plate counts from tissues of plants inoculated with Bacterium tabacum*

Dilution plate no.	Colonies of *Bact. tabacum* from tissues of—			
	Tobacco	Tomato	Cucumber	Bean
	Number	*Number*	*Number*	*Number*
1	418	39	93	35
2	379	24	91	36
3	78	2	49	11
4	19	1	3	4

The data in table 2 were obtained during a period of medium temperatures (65° to 75° F.). Plants were also inoculated and held at temperatures of 55° to 65°, with results indicating that the bacterium in plants other than tobacco can survive longer under these cooler conditions. On the other hand, during very warm weather, even after 3 weeks, it was often impossible to reisolate the organism from any plant except tobacco. It has been previously stated that some plants are much more sensitive to the action of the toxin than tobacco, and the bean is especially so. The sensitivity of the plant to the toxin, however, and the ability of the organism to establish itself and to multiply in the plant tissues seem to have no relation to each other. Thus from inoculations made at the same time on bean and tobacco the lesions on the bean leaves were much larger than those on tobacco, so that on the basis of symptoms one would classify the bean as highly susceptible and the tobacco as moderately susceptible. Consequently the general practice of measuring susceptibility of plants to a disease by the intensity of the symptoms produced appears to be misleading in the case of *Bacterium tabacum*. The bean, which is susceptible to the toxin, is a most unfavorable food plant for the bacteria (table 3), and hence the ability of this organism to establish parasitic relationships is dependent not on susceptibility of the plant tissues to its toxin, but rather on some unknown quality which favors the growth and multiplication of the bacteria.

TABLE 3.—*Dilution-plate counts from tissues of tobacco and bean 4 weeks after inoculation with Bacterium tabacum*

Dilution plate no	Colonies of *Bact tabacum* from tissues of	
	Tobacco	Bean
	Number	*Number*
1	([a])	16
2	([a])	5
3	1,000+	1
4	450	0
5	63	0
6	30	0

[a] Colonies too numerous to count.

FIELD EXPERIMENTS

INOCULATIONS

In order to study the occurrence of wildfire disease under field conditions, plantings of tobacco were made with a number of other crops intermingled. Some of the plants used in this manner were cantaloup, cucumber, bean, eggplant, pepper, tomato, sweetpotato (*Ipomoea batatas* (L.) Lam.), potato (*Solanum tuberosum* L.), watermelon (*Citrullus vulgaris* Schrad.), chrysanthemum (*Chrysanthemum coronarium* L.), snapdragon (*Antirrhinum majus* L.), sweet alyssum (*Alyssum maritimum* L.), coleus (*Coleus blumei* Benth.), and zinnia. To inoculate the plantings uniformly the bacteria were grown in liter flasks and the diluted cultures applied with an ordinary compressed-air sprayer. Inoculations were made during rains at intervals through the season, and infection consisting of a few scattering lesions was secured at various times on cucumber, cantaloup, bean, pepper, and zinnia (fig. 6). The difference between the response of these plants and that of tobacco, however, was twofold: (1) The number of lesions secured on tobacco was very much larger than on any other plant; (2) with tobacco there was a continuous spread of the disease from infected to healthy leaves; with other plants there were occasional lesions without subsequent spread.

ISOLATIONS

Isolations and dilution plates from the lesions secured in the field plots gave results comparable to those already described for the greenhouse inoculations, except that whereas the plates from tobacco spots usually gave practically a pure growth of *Bacterium tabacum*, similar plates from the lesions on other plants often gave a very mixed bacterial growth.

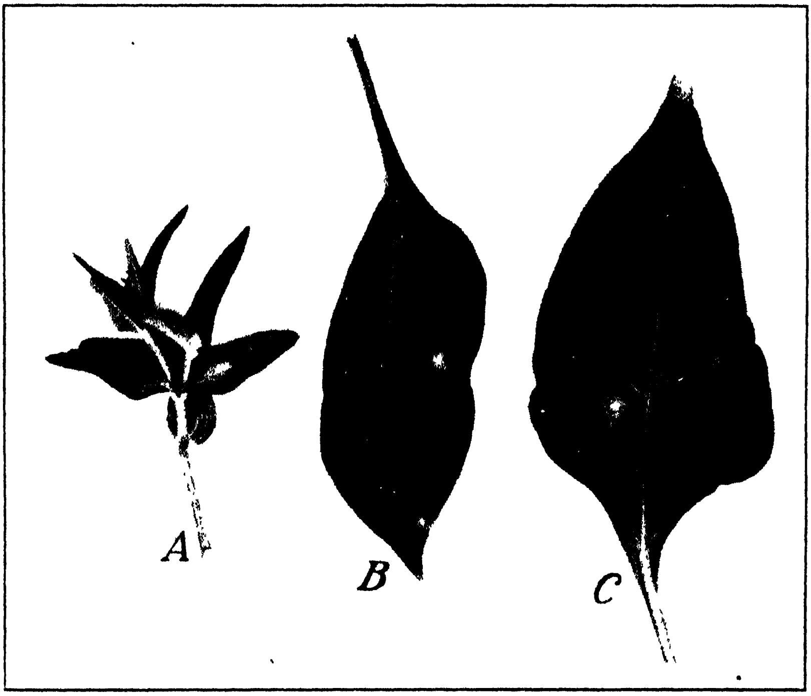

FIGURE 6 Zinnia (*A*) and pepper (*B* and *C*) leaf lesions resulting from field spray inoculations with *Bacterium tabacum*. Such occasional infections were secured with a variety of plants, and in some instances, at least, were not associated with wounds of any sort.

SUSCEPTIBILITY OF SPECIES OF NICOTIANA

During the course of these experiments many varieties of *Nicotiana tabacum* and certain other *Nicotiana* species were successfully inoculated with *Bacterium tabacum*. No attempt was made at this time to resurvey the field covered by Anderson (*1*) in his study of the susceptibility of *Nicotiana* species. The methods adopted in his inoculations obviated completely the possibility of misleading toxin effects.

DISCUSSION

The subject of toxins has received attention from plant pathologists in recent years, and the existence of toxic materials has been demonstrated in studies of certain wilt diseases by Young and Bennett (*14*), Fahmy (*4*), Goss (*5*), Rosen (*10*), and others. These results indicated that the fungi concerned produced poisonous substances which were the direct cause of the wilting, this view replacing the earlier conception of a mechanical plugging of the water-conducting tissue. These materials were mostly thermostable. In certain cases it was shown that the wilt-producing toxin of a particular parasite was effective in wilting other plants than the usual host. Likewise a given plant may be wilted by products from other fungi than the usual parasite. The general conception has been that these wilt-producing substances are probably poisonous growth products. The fact that plants have not been immunized against such toxic substances has also been cited by Picado (*9*) as a reason for not considering them to be true toxins. However, acquired immunity is unknown in plants, and this may be due to certain basic structural and functional differences between plants and animals rather than to the fact that plant parasites do not produce toxins.

With *Bacterium tabacum* and the wildfire disease of tobacco, the question of toxic action has assumed importance in studies of environmental relations, overwintering, and host range, because of the general practice of inoculation by wounding and of regarding the development of a characteristic lesion as proof of infection. Studies of the properties of the *Bact. tabacum* toxin indicated that it is present in very young cultures and hence is not a gradual accumulation of waste products. In fact, it is likely to disappear from old cultures. It is excreted by the bacteria. It has a specific action on chlorophyll tissue, with the death of other tissues following as a later development, and it is active in high dilutions. These properties indicate that it falls in the class of soluble exotoxins, and as such it differs distinctly from the exotoxins known to animal pathology. These latter give protein reactions, they are destroyed by heating and are precipitated by heavy metals, while the toxin of *Bact. tabacum* is not. In this connection it may be noted that there has been a question as to whether the animal-parasite exotoxins are actually proteinlike or are merely closely linked to protein, and hence whether a pure exotoxin would actually react like protein.

Proof of the pathogenicity of an organism is generally based on the well-known Koch postulates. Pathologists conducting studies of host range, however, have frequently regarded the production of typical disease symptoms after pure-culture inoculation as proof of a parasitic relationship, and this procedure was followed in some of the previous work with *Bacterium tabacum*. This practice rests on the assumption that the lesions must be the result of parasitic activity of the bacteria in the plant tissues. The writer's experiments show, however, that with the usual method of wound inoculation it was possible to produce characteristic wildfire lesions on the leaves of most of the plants tested, and that these results could be secured with solutions containing the bacterial toxin whether or not the bacteria were present. Similar tests with bacteria washed free from toxin, however, consistently produced typical wildfire lesions only on tobacco. Further investigations indicated that the degree of susceptibility to the toxin was a stable property varying widely with different species of plants. With the bean, a highly toxin-susceptible plant, the lesions ranged up to 1 inch in diameter and the dead central area was surrounded by a broad halo band. Lettuce (*Lactuca sativa* L.) was nearly nonsusceptible, and the inoculated leaves showed only faint halo spots. Tobacco proved to be only very moderately toxin-susceptible. The following explanation of the mechanics of these lesions is suggested.

The wound inoculations provided entrance into the leaf for a small amount of the toxic substance, which diffused out from the central point into the tissues. The leaf chloroplasts appeared to be most sensitive to the toxin action, and were destroyed first. The disintegration of the chlorophyll made the yellow carotene pigment visible, and hence a yellow halo spot was formed. With many plants this was the extent of the lesion, but with certain very susceptible plants the tissues in the center that received the most toxin were killed entirely, and the yellow halo then appeared around this central dead area. These lesions, therefore, were fundamentally chemical in nature and not a proof of parasitism, since various chemicals similarly pricked into leaves would result in producing lesions of various types, and differences in the susceptibility of species could doubtless be established. Final proof that susceptibility to the *Bacterium tabacum* toxin and susceptibility to parasitic attack by the bacteria are not correlated is supplied by the fact that the wound inoculations with toxin and bacteria used separately give very different results, the conclusion being that many plants are susceptible to the action of the toxin while only tobacco is susceptible to attack by the bacteria.

Infection as it occurs under natural conditions, of course, is not complicated by toxin effects, such as occur in conducting wound inoculations with pure cultures. In nature the bacteria are spattered from leaf to leaf by rain, and generally penetrate the uninjured leaf surface. It is significant that under such natural conditions *Bacterium tabacum* has not been found infecting any plant except tobacco. Of previous investigators, only two have observed natural infection on plants other than tobacco. Wolf (*12*) observed lesions on cowpeas (*Vigna sinensis* (L.) Endl.) which appeared to start around insect punctures. These lesions remained small, and his conclusion was that the organism was "certainly not actively parasitic." Chapman and Anderson (*2*) observed lesions on tomato leaves which appeared to have started around injuries.

A second line of evidence considered by pathologists in deciding on host relationships concerns the demonstration of the presence or absence of viable pathogenic bacteria in the plant tissues after successful inoculations as judged by the production of disease symptoms. This reisolation and identification of the organism constitutes the concluding steps as prescribed by the Koch postulates.

In these experiments, the organism was reisolated from a variety of plants. Reisolation was most readily accomplished in the greenhouse experiments, since in the field the lesions on plants other than tobacco were soon contaminated by saprophytic bacteria. It was also usually necessary with these other plants to make the reisolations no later than 4 weeks after the inoculations. These reisolations did not always consist of merely removing the bacteria from the point at which they were originally placed, as, in the case of the bean, the leaf tissue around the point of puncture was killed, and yet the bacteria could be recovered from the inner edge of the chlorotic area. The pathogenicity of the recovered bacteria was readily demonstrated. Evidence of this sort might be accepted as definite proof that the plants concerned were host plants, and, if this point of view were taken, then the list of host plants for *Bacterium tabacum* could be expected to include the greater part of the plant kingdom, since plants nonsusceptible to the toxin are the exception, and the bacteria usually remain alive in the various plant tissues for some time.

As opposed to this point of view, the following arguments may be advanced: (1) The production of typical leaf lesions by wound inoculations with cultures containing toxin cannot be considered as proof of parasitism on the part of the organism, since these lesions were obtained with the toxin whether or not living bacteria were used. (2) The significance of the presence of the bacteria in the tissues of such plants as bean, tomato, and cucumber, after wound inoculation, would appear to depend on further information as to the state of their activity in the tissues of these plants. Thus the bacteria are known to be able to remain alive in limited numbers for a period of months in diseased leaves that are air-dry, and even on such materials as the wood or cloth used in the building of plant beds, and yet it would not be suggested that under these conditions they were active as parasites. It was shown by dilution-plate counts that after wound inoculations the bacteria in the leaf tissues decrease rapidly in all plants except tobacco. Consequently after a few weeks there may be 1,000 times as many bacteria in the tobacco tissues as in bean tissues, for example, and later all the bacteria may be dead in the bean leaves but they still remain abundant in the tobacco. A reasonable conclusion would appear to be that in the dead or weakened tissues that result from the action of the toxin some bacteria are able to live and perhaps secure food and reproduce for a short time, without, however, being parasitic in the sense that they are on tobacco.

The view that parasitic organisms are quite incapable of existing in tissues of plants other than their host plants is further weakened by the results secured by Young (*15*), who found that under favorable artificial conditions many parasitic and saprophytic fungi were able to invade to a limited degree the tissues of numerous plants, but that under normal conditions such infections did not occur. On the basis of his ability to produce infection and to reisolate, he was able to list some 200 new diseases. Johnson (*6*), in similar experiments, was able to demonstrate that *Colletotrichum circinans* (Berk.) Vogl. could invade the tissues of 20 out of 22 species of plants tested, and could be reisolated after surface sterilization, yet so far as known this fungus under natural conditions attacks only species of *Allium*.

Host range is a matter of considerable practical importance because plants listed as hosts are usually regarded as liable to attack by the disease under field conditions, and hence they offer possible means whereby the disease may be carried over winter or be introduced into new areas. It is usually assumed that host plants provide a favorable medium for the multiplication and spread of the parasite. With the exception of tobacco, none of the plants studied showed indications of being liable to infection under natural conditions or of providing favorable conditions for the multiplication and spread of the bacteria;

consequently it is concluded that only species of *Nicotiana* should be regarded as hosts of *Bacterium tabacum*.

SUMMARY

Bacterium tabacum Wolf and Foster, the cause of the wildfire disease of tobacco, secretes a powerful toxin. This toxin passes readily through the ordinary filters, is not precipitated by alcohol or by neutral calcium or lead acetates, is not removed from solution by boiling with bone black, is not affected by formaldehyde, mercuric chloride, or acids, and is thermostable. It is quickly inactivated by dilute alkalies.

When pricked into leaves this toxin destroys the chlorophyll and produces the halo lesions typical of the wildfire disease. The leaves of many plants are susceptible to the action of the toxin, some being much more sensitive than tobacco.

Inoculation experiments with (1) toxin alone, (2) toxin plus bacteria, and (3) bacteria alone yielded large conspicuous lesions from 1 and 2 on a wide range of plant species. With 3 the results were either no lesions or else lesions that remained small and undeveloped in the case of all plants tested, with the exception of species of *Nicotiana*. Tobacco leaves inoculated with toxin-free bacteria developed lesions more slowly than those inoculated with a combination of toxin and bacteria, but the end results were the same.

Poured-plate isolations from many inoculations have shown that in plants other than tobacco the bacteria survive for a few weeks but gradually become fewer and finally die out entirely. In tobacco leaf tissues they continue to live and multiply.

Many plants on which the disease does not usually occur and in the tissues of which the bacteria cannot indefinitely persist may be successfully inoculated as judged by (1) the production of typical wildfire leaf lesions, (2) the ability to reisolate the organism, and (3) positive results from reinoculation. Hence the advisability of unqualified acceptance of the Koch rules of proof is questioned for diseases such as the one under discussion.

It is considered that only species of *Nicotiana* should be regarded as true host plants for *Bacterium tabacum*.

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CHARACTERISTICS OF THE PIROPLASMS BABESIA ARGENTINA AND B. BIGEMINA IN THE UNITED STATES[1]

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PURPOSE OF INVESTIGATION

The purpose of the present investigation was to determine (1) what species of *Babesia* occur in this country, (2) what their morphological and physiological characters are, and (3) whether there is any variation in their reaction to trypan blue, which thus far has been the most promising means of treating clinical cases of babesiasis. In order to ascertain whether *B. argentina* is a valid species, the morphological characters of piroplasms occuring in Europe, Africa, and South America were also studied.

REVIEW OF LITERATURE

VALIDITY OF PROPOSED GENERA AND SPECIES

In 1893 Smith and Kilborne (*13*)[2] stated that the shape of piroplasms resembles that of apple seeds. Subsequent investigators have shown that the piroplasm nucleus is composed of chromatin particles occurring in more than one body; in other words, the chromatin material is scattered. Up to the present time nothing else of morphological importance has been added. Therefore, there appear to be no valid criteria for a number of genera that have been proposed in the family Babesiidae Poche, 1913, and the writer concurs in the views of Wenyon (*15*) and Reichenow (*10*) that only one generic name, *Babesia* Starcovici, 1893, is valid, and that the following names are synonyms: *Piroplasma* Patton, 1895; *Nicollia* Nuttall, 1908; *Nuttallia* França, 1909; *Smithia* França, 1909; *Rossiella* Nuttall, 1912; *Microbabesia* Sohns, 1918; *Babesiella* Mesnil, 1919; and *Françaiella* Yakimoff, 1926. Under these names are reported parasites of bovines, ovines, equines, canines, and rodents.

The occurrence in Argentina of two species of *Babesia*, *B. bigemina* and *B. argentina*, was first reported by Lignières (*5*). In material collected from cases of piroplasmosis in Louisiana, the writer (*8*) noted that the piroplasms were scanty in the peripheral blood; that they were abundant post mortem in the organs, minute in size when compared with other piroplasms known to be *B. bigemina*, and that quadruple infections of erythrocytes were common. Dennis,[3] of the University of California, noted a resemblance to the genus *Nuttallia* and regarded the species as different from *B. bigemina*. Becker,[3] of the Iowa State College of Agriculture, noted that the angle between the longitudinal axes of members of the intraglobular couple was usually about 180°, whereas in *B. bigemina* which he obtained from Texas it was seldom greater than 60°. Therefore, Becker and Rees

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[1] Received for publication Nov. 13, 1933; issued May 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 437.

[3] Personal communication.

(*2*) reported the occurrence in Louisiana of a species of *Babesia* (syn., *Piroplasma* Patton, 1895) in addition to the well known *B. bigemina*. This other species, reported by Rees (*8*) as *B. bigemina*, has been determined as *B. argentina* Lignières, 1903.

It appears advisable to recognize *Babesia bigemina* and *B. argentina* as valid species, although Wenyon (*15*), Schilling (*11*), and Reichenow (*10*) do not concur in this view. *B. bovis* Starcovici, 1893, is commonly recognized as a valid species; it is a very small piroplasm like *B. argentina*, and resembles the latter also in that the angle between longitudinal axes of the couple of intraglobular parasites is usually about 180°, but it differs from *B. argentina* in that it is located in the margin of the infected erythrocyte, whereas *B. argentina* is located in the center. As is shown by the data of the present paper, *B. berbera* Sergent and collaborators, 1924, (*12*) resembles *B. argentina*. Yakimoff and collaborators (*16, 17, 18, 19*) have proposed several new species for forms occurring in northern Europe and in Asia that are practically indistinguishable from *B. argentina*.

PIROPLASMS STUDIED BY SMITH AND KILBORNE

The report of Smith and Kilborne (*13*) carries adequate evidence that they were dealing with both *Babesia bigemina* and *B. argentina*. They show in plate 7, figure 2, drawn from a capillary in the kidney, what is clearly a pure infection with *B. argentina*; the parasites are uniform in size, being not more than 2½μ long, which is much too small for *B. bigemina*. In plate 4, figure 4, and plate 6, figures 1 and 2, they show the commonly occurring quadruple infection of erythrocytes which characterizes an infection with *B. argentina*. They report very severe infections and state (*13, p. 61*) that "a long search is necessary before one (parasite) is brought into view", and they say further in the same paragraph: "When present in considerable numbers in the blood the infected corpuscles usually appear in groups in the field of the miscroscope as is shown in the figure referred to (pl. 5, fig. 2), and not uniformly distributed." In further explanation of the figure they state: "The appearance of the infected corpuscles in groups * * * was especially marked in this animal." Lignières (*5*) pointed out that the latter phenomenon is a characteristic of infections produced by *B. argentina* but not of infections produced by *B. bigemina*. Although it has been copied in text books as a figure of *B. bigemina*, this illustration of Smith and Kilborne (pl 5, fig. 2) is clearly a figure of *B. argentina*. The following statement occurs in their paper (*13, p. 61*):

> With only 1 or 2 per cent, or even 10 per cent, of infected corpuscles in the circulating fluid, it would be difficult to account for the enormous daily losses of blood corpuscles in the acute fever. The difficulty is cleared up by sacrificing an animal in the earlier days of the fever and examining the internal organs for infected corpuscles. Large numbers of parasites are found within corpuscles in the capillary blood of congested areas, such as those of the heart muscle and of the omentum.

The foregoing citations are good descriptions of a typical infection with *Babesia argentina*, but not of one with *B. bigemina*.

Intraglobular forms of the parasite are illustrated in Smith and Kilborne's paper (*13*) in text figure 3, which shows that the angles between the longitudinal axes of the couples are about 150°, 160°, and 180°, respectively; the organisms figured are *Babesia argentina*.

On the other hand, in plate 5, figure 3, a smear of peripheral blood, the organisms are *B. bigemina;* a solitary spindle-shaped form is about 7μ long, which is longer than the diameter of the normal bovine corpuscle, whereas the other parasites in grouped couples are 4μ long. The unstained parasites figured in plate 8, figures 1–5, are *B. bigemina.* Though other pertinent citations might be furnished, the data presented by Smith and Kilborne show adequately that two species of *Babesia*, *B. bigemina* and *B. argentina*, were seen and described by them under the name *Pyrosoma bigeminum.*

EFFECT OF TRYPAN BLUE ON PIROPLASMS

A number of prominent veterinarians who have large cattle practices in Louisiana have told the writer they they do not use trypan blue in cases of bovine piroplasmosis. These practitioners stated that whereas in certain outbreaks clinical cases were cured by intravenous injections of the drug, in other outbreaks it had no apparent effect.

Trypan blue was found by Nuttall and Hadwen (*7*) and by Theiler (*14*) to be a specific in infections with *Babesia canis* (Piana and Galli-Valerio, 1895) and *B. bigemina*, but was found by Brumpt (*3*) to have no marked therapeutic action in infections with *B. argentina.*

MATERIAL

The material used in the present investigation was obtained from the following sources: (1) Smears from the heart of a cow that was in a herd of purebred Holstein-Friesians, at Lafayette, La., in which 12 out of about 13 cases of infection with *Babesia argentina* proved fatal; (2) a strain of this species of *Babesia* obtained by infecting another cow at the Jeanerette station with the progeny of a tick, *Boophilus annulatus* (Say, 1821), Curtice, 1891, taken from one of these cows; (3) 35 other cases of infection with *B. argentina*, all occurring in Louisiana; (4) 8 cases of infection with a strain of *B. bigemina* shipped to Jeanerette, La., in blood from Texas; (5) 2 naturally occurring Louisiana cases involving *B. bigemina;* and (6) smears of the following piroplasms which were sent to the writer through the courtesy of the investigators named: *Babesia bovis* from Dr. R. Wetzel of the Tierärztliche Hochschule, Hannover, Germany; *Babesiella berbera* from Dr. Sergent, of the Institut Pasteur d'Algérie; *Babesiella minor* Quevedo, 1918, and *Babesia bigemina* from Dr. F. Rosenbusch, of Buenos Aires, Argentina. The writer believes that all these forms belong in the genus *Babesia* and considers *Babesiella minor* a synonym of *Babesia argentina.*

MORPHOLOGICAL CHARACTERS OF THE PIROPLASMS

BIOMETRY

Table 1 shows that *Babesia bigemina* is longer than *B. argentina*, the latter being about the same length as *B. berbera*, and, furthermore, that *B. bovis* is the smallest of the four species. The various forms of *Babesia* occurring in the peripheral blood have mean lengths that vary from 2.26 ± 0.04μ (smear of *B. bigemina* from Argentina) to 5.00 ± 0.05μ for *B. bovis.* There were three length groups, about 4, 3, and 2.25μ, respectively, with *B. bigemina* in the first group, *B. argentina* and *B. berbera* in the second, and *B. bovis* in the third. The ranges in length (table 1) were from 2.5 to 5.5μ for *B. bigemina*, 2 to 4.5μ for *B. argentina*, 2 to 5μ for *B. berbera*, and 2 to 3μ for *B. bovis*, so that, except for *B. bovis*, there was almost complete overlapping. In smears of *B. bigemina* from Argentina the parasites were longer than in smears of the same species from Texas, the means being 5.00 ± 0.05μ and 4.02 ± 0.04μ. This fact supports the statements which are found generally in the literature that discontinuous variations occur between different strains in the same species.

Table 1 shows that *B. bigemina* occurring in smears made from the heart is smaller than in those made from the peripheral blood, the mean lengths being 4.02 ± 0.04μ and 3.70 ± 0.04μ; this applies also to *B. argentina*, with corresponding lengths of 3.14 ± 0.04μ and 2.75 ± 0.03μ. However, in each species these differences of less than 1μ were not so great as those shown in the drawings by Smith and Kilborne (*13*) all of which were supposed to be of *B. bigemina.*

TABLE 1.—*Length of specimens of Babesia and magnitude of angle of the intraglobular couple*

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
BABESIA BIGEMINA				
Peripheral blood (Texas)	2.5	8	20	40
	3.0	18	30	34
	3.5	33	45	13
	4.0	34	60	12
	4.5	17	90	37
	5.0	38	120	4
	5.5	2	150	6
			180	4
Mean or total	4.02±0.04	150	57.0	150
Heart blood (Texas)	2.5	12	20	12
	3.0	11	30	35
	3.5	21	45	13
	4.0	41	60	8
	4.5	10	90	26
	5.0	5	150	3
			180	3
Mean or total	3.70±0.04	100	56.8	100
Peripheral blood (Argentina)	4.5	6	20	14
	5.0	13	30	5
	5.5	6	45	2
			60	2
			90	2
Mean or total	5.00±0.05	25	32.8	25
BABESIA ARGENTINA				
Peripheral blood (Louisiana)	2.0	3	20	8
	2.5	23	30	9
	3.0	31	45	6
	3.5	31	60	6
	4.0	10	90	21
	4.5	2	120	7
			150	18
			180	25
Mean or total	3.14±0.04	100	109.9	100
Heart blood (Louisiana)	2.0	9	20	3
	2.5	44	30	4
	3.0	36	45	6
	3.5	9	60	7
	4.0	2	90	14
			120	7
			150	20
			180	39
Mean or total	2.75±0.03	100	129.9	100

TABLE 1.—*Length of specimens of Babesia and magnitude of angle of the intraglobular couple*—Continued

BABESIA ARGENTINA (SYN., BABESIELLA MINOR)

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
Peripheral blood (Argentina)	2.0	3	20	1
	2.5	8	30	3
	3.0	10	90	6
	3.5	3	120	1
	4.0	1	150	7
			180	7
Mean or total	2.82±0.07	25	123.2	25

BABESIA BERBERA (SYN., BABESIELLA BERBERA)

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
Peripheral blood (Algeria)	2.0	9	20	6
	2.5	14	30	9
	3.0	15	45	1
	3.5	6	60	3
	4.0	4	90	13
	4.5	1	120	6
	5.0	1	180	12
Mean or total	2.89±0.06	50	93.3	50

BABESIA BOVIS

Kind of smear and source	Length		Magnitude of angle	
	Microns	Frequency	Degrees	Frequency
Peripheral blood (Germany)	2.0	13	20	1
	2.5	11	30	1
	3.0	1	45	2
			90	4
			120	1
			150	3
			180	13
Mean or total	2.26±0.04	25	136.4	25

With respect to the angle occurring between the longitudinal axes of the members of the couple in piroplasms, the writer's data show two main groups as follows: (1) *B. bigemina*, with a mean angle of 57° in the Texas strain and 32.8° in the Argentine strain; and (2) *B. argentina* from Louisiana and Argentina, *B. berbera* (syn., *Babesiella berbera*), and *Babesia bovis*, in which species the range of the mean angle was from 93.3° for *B. berbera* to 136.4° for *B. bovis*. It will be noted from table 1 that for all the species in the second group, except *B. berbera*, the mode was about 180°. The latter species was bimodal, i.e., at 90° and 180°. A difference of 20° between the means of *B. argentina* in the peripheral blood and in the heart blood shows that in a given species this character is subject to wide variations. In living couples of *B. argentina*, the writer has observed rapid rotations from an angle of 90° to one of 270°. In fixed material, the maximum figure that can be read is 180° and, therefore, the statistics may not show the real differences between the angles of the two species. Table 1 shows that in the four species of piroplasms the range of variation was from 20° to 180°, i.e., there is complete overlapping. Although in the smaller *Babesia*, i.e., *B. argentina*, *B. berbera*, and *B. bovis*, the mean angle is greater than in *B. bigemina*, the limits of variation in all four species are the same so far as the angle is concerned.

CYTOLOGY

Drawings, all on the same scale, of *Babesia bigemina* from Texas, *B. argentina* from Louisiana and Argentina, *B. berbera* from Algeria, and *B. bovis* from Germany are presented in figure 1. These drawings show in general the same features as those of previous investigators; there are no morphological criteria other than size, magnitude of angle, and position within the erythrocyte for the determination of species.

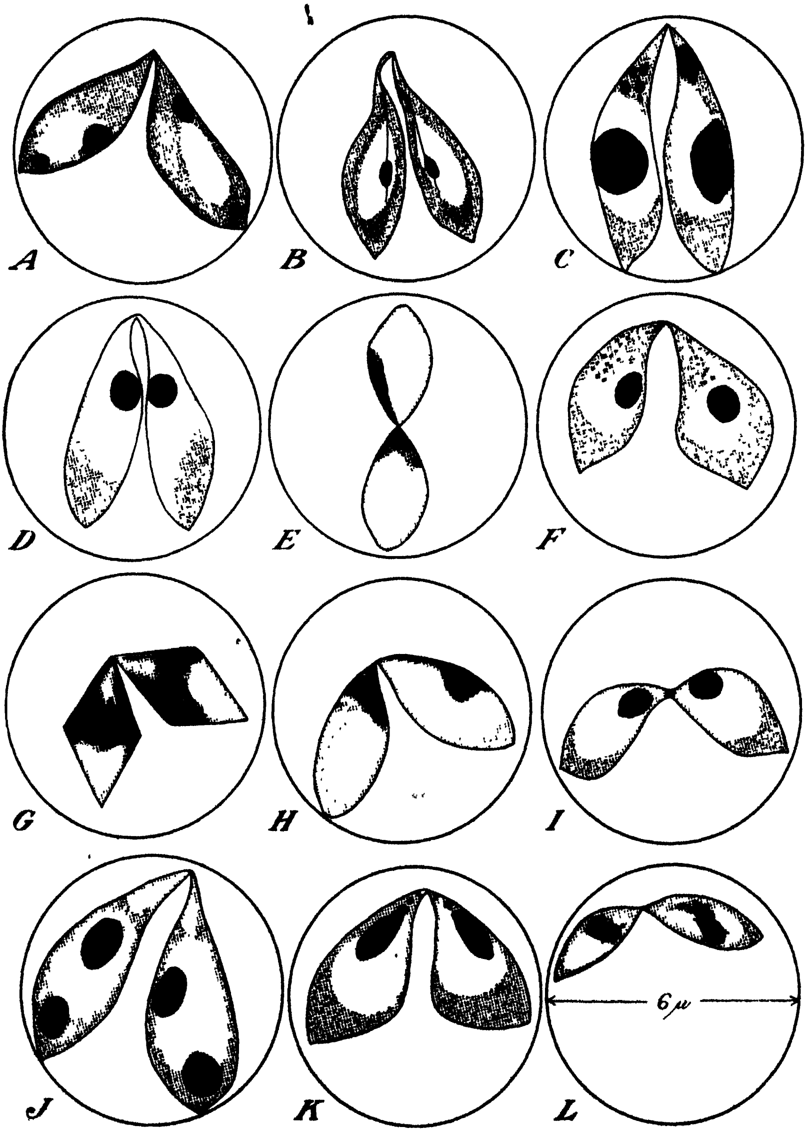

FIGURE 1.—Camera lucida drawings of four species of *Babesia: A, B, C, B. bigemina* from peripheral blood; *D, B. bigemina* from heart blood; *E, F, B. argentina* from peripheral blood; *G, H, B. argentina* from heart blood; *I, B. argentina* (syn., *Babesiella minor*) from peripheral blood; *J, K, Babesia berbera* (syn., *Babesiella berbera*) from peripheral blood; *L, Babesia bovis* from peripheral blood. *B*, from a preparation stained with iron haematoxylin; all others with Giemsa's stain.

Typical forms of *Babesia bigemina* are shown in figure 1, *A*, *B*, and *C*. Figure 1, *B*, from a preparation which was stained in iron haematoxylin, shows a fine fibril such as was described by Dennis (*4*) as the rhizoplast. It could not be found in preparations in which other stains were used. The writer was unable to differentiate the structure which Dennis (*4*) described as the nuclear membrane. However, there was usually a halo about the large chromatin granule.

Figure 1, *D*, illustrating the appearance of *Babesia bigemina* from the heart, shows 1 mass of chromatin in the nucleus, not 2 or 3 masses such as occur in parasites from peripheral blood; this characteristic was general in the writer's material.

Figure 1, *E* to *H*, illustrates *Babesia argentina* from Louisiana, *E* and *F* being from peripheral blood and *G* and *H* from heart blood. *G* is similar to the couple of spindle-shaped parasites shown in text figure 3 of Smith and Kilborne's (*13*) paper. These authors mentioned spindle forms as being typical of *B. bigemina*. The writer has found spindle forms consistently in *B. argentina* but never in *B. bigemina*.

I, in figure 1, is *Babesia argentina* (syn., *Babesiella minor*) from Argentina. *J* and *K* are *B. berbera* (syn., *Babesiella berbera*) from a preparation by the Institut Pasteur d' Algérie. The size of *B. berbera* in *J* is similar to that of *B. bigemina;* although large parasites were rare in *B. berbera*, their occurrence was demonstrated by statistics also. *B. berbera* (*K*) is indistinguishable from *B. argentina* (*F*). Figure 1, *L*, shows that *B. bovis*, which was received from Hannover, Germany, was easily distinguishable from the three other species both by its smaller size and by its marginal position within the erythrocyte.

PHYSIOLOGICAL CHARACTERS OF THE PIROPLASMS

NUMBER DURING LIFE IN THE PERIPHERAL BLOOD AND POST MORTEM IN THE ORGANS

In the writer's cases of *Babesia argentina* infection, the parasites were scanty in the peripheral blood, agreeing in this respect with descriptions of infection produced by this species in other parts of the world. On September 18, 1931, blood was drawn from a "carrier" at Kaplan, La., defibrinated, and then injected intravenously into two Brahman-Hereford cows, nos. 77 and 78, at Jeanerette, La. Both cows reacted on the sixth day, no. 77 died on the eleventh day and no. 78 on the twelfth day; each case had haemoglobinuria for 3 days prior to death. During this time infected corpuscles were scanty and hard to demonstrate in the smears. In occasional smears, however, they were plentiful; when plentiful they occurred in groups. Figure 2 shows the occurrence of *B. argentina* in 15 out of 16 corpuscles in the blood of cow 77.

It has been stated elsewhere in this paper that whereas *Babesia argentina* was scanty in the peripheral blood it occurred abundantly in smears made from the organs. Figure 3 represents 16 corpuscles in a capillary of the brain of cow 77; 15 of the corpuscles were parasitized; in one case there was a quadruple infection, in another a triple infection, and there was one couple of extraglobular piroplasms.

In *Babesia bigemina* infections the parasites were numerous in the peripheral blood and were uniformly distributed, never occurring in groups as in the case of infection produced by *B. argentina*. Capillaries which were congested with infected erythrocytes were not found post mortem in the organs, not even in the brain. This was in marked contrast to the condition occurring in fatal cases produced by *B. argentina*.

The foregoing data indicate the following things: (1) The principal seat of multiplication of *Babesia argentina* is in the capillaries, particularly in those of the internal organs, and when released into the peripheral circulation *B. argentina* occurs in clusters and is not uniformly distributed; (2) infections with *B. bigemina* are heavy in the peripheral blood; (3) in the peripheral blood *B. bigemina* does not occur in groups as does *B. argentina*; (4) the infections with *B. bigemina* in the capillaries of the internal organs are light as compared with those of *B. argentina*.

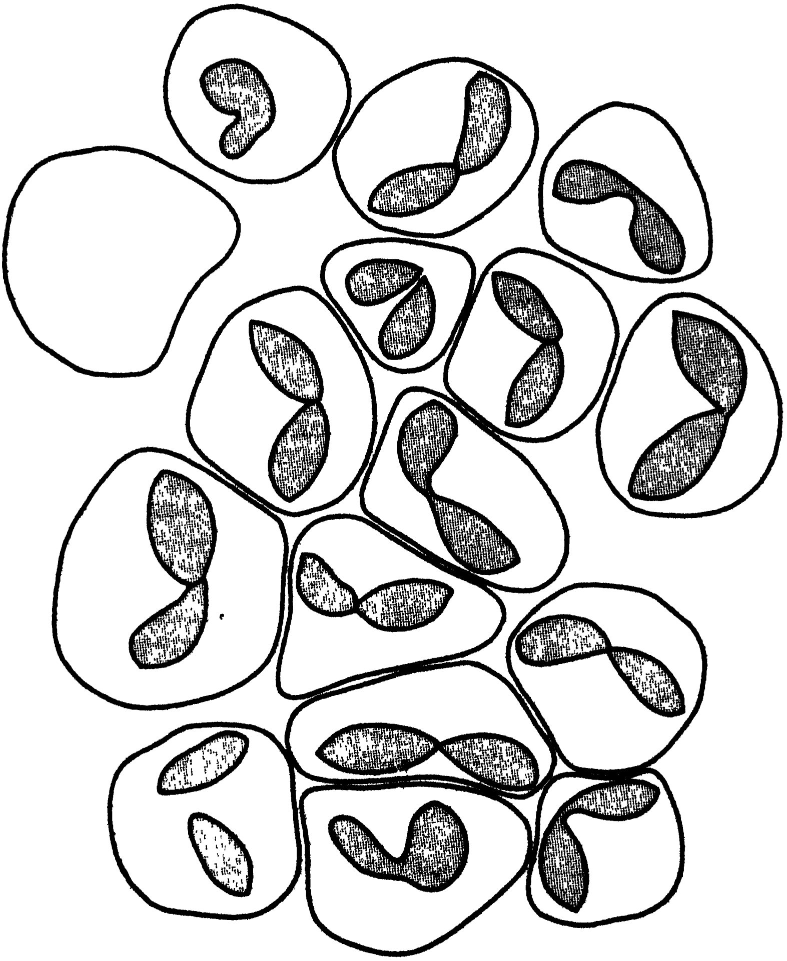

FIGURE 2.—Camera lucida drawings showing the occurrence of *Babesia argentina* in 15 out of 16 corpuscles of the peripheral blood; live blood from cow 77.

BEHAVIOR IN CULTURE

By the method of Bass and Johns (*1*) the writer was able to cultivate *Babesia argentina* for 96 hours, but he did not succeed in establishing subcultures. *B. bigemina* could not be cultivated, the parasites becoming rounded up within several hours after transfer to the culture tubes and disappearing within 36 hours.

REACTIONS TO TRYPAN BLUE

A case of infection with *Babesia bigemina* was discovered in bull 70 on April 2, 1932, at the experimental barn. Rees (*9*) has reported this case as one of accidental transmission by an infected lancet. A count on April 2 showed 120 infected erythrocytes per 1,000. This bull weighed 650 pounds and was treated by intravenous injection with 600 mg of trypan blue in 500 cc of physiological saline. In smears which were taken 4 hours after treatment there was only an occasional parasite, and on the following morning there were none. The prognosis on the morning of April 4 was favorable, but the bull was accidently killed by a drenching procedure. Smears from the heart, brain, spleen, and liver were all negative for piroplasms.

Bull 71 had a very severe case of infection with *Babesia bigemina* on August 2, 1932, the urine being colored a deep blood red, and although no counts were made, the infection in the peripheral blood was heavy. This bull weighed about 800 pounds. It was injected with 1 g of trypan blue at 4 p.m. by the same method as with bull 70. At 10 p.m. there were very few parasites in the smears and these appeared to be poorly defined and did not stain as do normal piroplasms. The smears that were made the next morning were negative. Within 24 hours after treatment haemoglobinuria disappeared; the bull made a rapid recovery.

The writer has been favored with the cooperation of H. Laughlin, a veterinarian, in treating nine cases of infection with *Babesia argentina* that occurred in his local practice from March 2 to August 31, 1932, microscopic diagnoses having been made by the writer. Details concerning two of these cases were as follows:

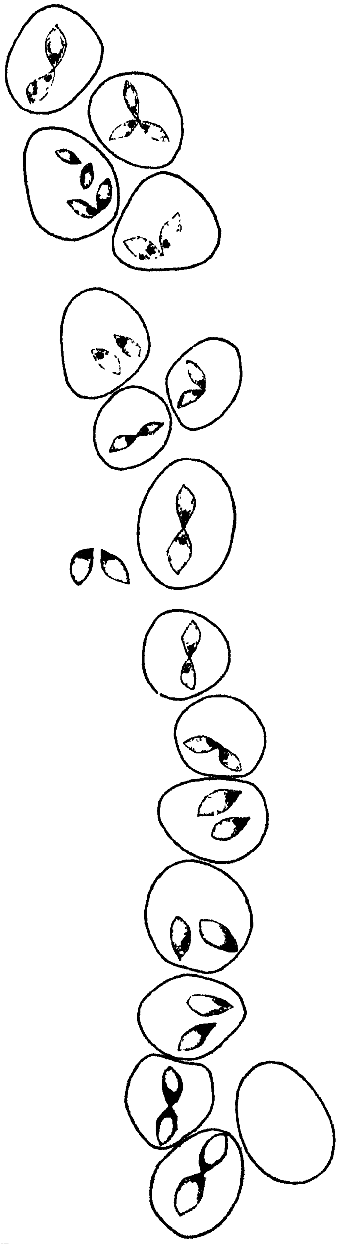

FIGURE 3.—Camera lucida drawings from a capillary in the brain of cow 77, showing 16 corpuscles, 15 of which were parasitized with *Babesia argentina;* also 1 pair of extraglobular corpuscles.

A purebred Jersey bull weighing about 1,500 pounds had haemoglobinuria and a temperature of 107° F. on July 13, 1932. Five smears showed *Babesia argentina;* in one smear the parasites were fairly numerous. At 9 a.m. the bull was injected intravenously with about 2 g of trypan blue; at 9 p.m. it was in extremis. At 5:30 a.m. on July 14, it was dead. While the body temperature was still near normal, smears of the heart, brain, and spleen were made, and these showed very heavy infections with *Babesia argentina*. The organisms were apparently rounded up but stained normally.

A grade Jersey steer weighing about 600 pounds was observed on August 31, 1932, at 11 a.m. and showed the same symptoms and parasites as the Jersey bull. It was injected intravenously at 12 m. with 3 g of quinine hydrochloride in 500 cc of normal saline, and at 1 p.m. with 1 g of trypan blue. On September 1, its condition was unchanged. On September 2, at 8 a.m., it was dead. Necropsy results were the same as in the case of the Jersey bull.

All seven of the remaining cases of *Babesia argentina* were treated with trypan blue only. One was examined post mortem on August 27, about 1 hour after death. This case had received two intravenous injections of trypan blue, one 72 hours before death and the other 48 hours before death. Smears of the organs showed numerous parasites apparently normal when compared with those in untreated fatal cases. Of the other six cases, not examined post mortem, three died within 24 hours, another within 48 hours, and the remaining two within 96 hours after treatment.

SUMMARY

A species of *Babesia* occurring in the United States has the following characters: (1) Minute size, (2) scanty occurrence in the peripheral blood although occasional occurrence in the field of the microscope in groups, (3) heavy infection in the internal organs, where quadruple infections of erythrocytes are common, and (4) the common occurrence of an angle of about 180° between the longitudinal axes of the two members of the intraglobular couple. On the basis of the above characters this species has been determined as *Babesia argentina* Lignières, 1903. The author's investigation has confirmed the above-mentioned characteristics.

Babesia bigemina is larger than *B. argentina*, infections are heavy in the peripheral blood and scanty in the internal organs, and the angle between the longitudinal axes of the members of the intraglobular couple is usually less than 60°.

The present investigations indicate that the piroplasms described and illustrated by Smith and Kilborne in 1893 included *B. argentina* as well as *B. bigemina*, although these workers did not differentiate the two species.

The mean length of a Texas strain of *B. bigemina* was 4.02 ± 0.04μ and that of an Argentine strain was 5.00 ± 0.05μ; the mean lengths of a Louisiana strain and an Argentine strain of *B. argentina* were 3.14 ± 0.04μ and 2.82 ± 0.07μ, respectively; that of an Algerian strain of *B. berbera* was 2.89 ± 0.06μ; and that of a German strain of *B. bovis* was 2.26 ± 0.04μ.

B. bigemina and *B. argentina* in the peripheral blood were larger than in the heart blood, but the difference in each case was smaller than 1μ, and less than that shown between peripheral blood forms and heart forms in the figures of Smith and Kilborne.

The magnitude of the mean angle formed by the intraglobular couple in piroplasms was as follows: (1) Argentine strain of *B. bigemina*

32.8°, (2) Texas strain of *B. bigemina*, 57°, (3) Argentine strain of *B. argentina* (syn., *Babesiella minor*), 123.2°, (4) Louisiana strain of *B. argentina*, 109.9°, (5) Algerian strain of *B. berbera*, 93.3°, and (6) German strain of *B. bovis*, 136.4°.

The writer's drawings of *B. bigemina* show this piroplasm in the heart blood with only one mass of chromatin but in the peripheral blood with more than one mass.

Spindle-shaped forms have been shown in the present paper to characterize *B. argentina* and not *B. bigemina*, contrary to the findings of Smith and Kilborne (*13*).

No significant morphological differences were detected between *B. argentina* and *B. berbera*, nor between a Louisiana strain and an Argentine strain of *B. argentina* (syn., *Babesiella minor*), but *B. bovis* was distinguishable from both of the above-named species by its smaller size and its marginal position within the erythrocyte.

B. argentina was cultivated for 96 hours in vitro; *B. bigemina* could not be cultivated.

In agreement with the results of previous investigators it was found that *B. bigemina* was killed by intravenous injections of trypan blue, but *B. argentina* was not demonstrably affected.

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THE CALCIFYING PROPERTIES OF GREEN, ARTIFICIALLY DRIED, AND SUN-CURED PASTURE HERBAGE[1]

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INTRODUCTION AND REVIEW OF PREVIOUS INVESTIGATIONS

The potency of the antirachitic vitamin in green forage has been a subject of considerable controversy. The difference of opinion regarding the antirachitic or the calcifying properties of green feeds is probably due to the difficulty in demonstrating through biological tests the small amounts of such properties contained in green feeds. Little experimental work has been done to determine the calcifying properties of pasture herbage, although considerable work has been done on other classes of green materials used in human consumption.

Chick and Roscoe (*3*)[3] demonstrated a slight but definite antirachitic value in summer-grown spinach, but failed to observe any antirachitic value in spinach grown in the open during winter, spring, or fall. Later Roscoe (*11*) reported a slight effect upon calcification in rats produced by the addition of 10 percent of green summer-grown spinach leaves to the experimental ration. Shipley, Kinney, and McCollum (*15*) found that the ether extract of 250 g of alfalfa leaves or clover blossoms, when mixed with a kilogram of the basal ration, caused healing of rickets in rats. However, they were unable to note an antirachitic action of ether extracts of similar amounts of dry spinach, brussels sprouts, celery, or cabbage. Bethke, Kennard, and Kick (*1*) found that leg weakness in chickens was not prevented by incorporating 18 percent (dry-matter basis) of fresh green red clover in the basal ration.

Research by Russell (*12*) showed that artificially dried alfalfa leaves possessed only a small amount of vitamin D, but that alfalfa dried in the sun was higher in its antirachitic action. This finding was confirmed by Steenbock and his coworkers (*17*), who concluded that the antirachitic properties of hays are related to their exposure to sunlight. Recently Smith and Briggs (*16*) presented further evidence to show that the antirachitic value of alfalfa was dependent upon its exposure to sunshine. The alfalfa that was cured in the absence of sunlight was deficient in the antirachitic factor.

In studies with dairy cattle, Hart and his coworkers (*6*, *8*) concluded that there was apparently enough of the antirachitic factor present in a ration made up of 40 pounds of green grass, 30 pounds of silage, and 14 pounds of grain when fed to cows producing from 40 to 60 pounds of milk per day, to maintain calcium equilibrium provided

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[2] The writers are indebted to Harvey Murer and Harold Gerritz, who were responsible for the analytical work, and to M. S. Grunder, who supervised the artificial drying of the herbage.

[3] Reference is made by number (italic) to Literature Cited, p. 445.

the calcium and phosphorus intake was sufficiently high. Calcium equilibrium was established when CaO was fed at a rate of approximately 1.5 percent of the daily ration. This was the case whether the animals were maintained out of doors or in stables without direct sunlight. Hart and others (*7*) failed to produce favorable calcium assimilation in heavy-producing cows that were given irradiated yeast, potent in vitamin D. They therefore concluded, as reported by Clark (*5*), that the ability of fresh green grass to cause calcium assimilation in milking cows was probably due to some factor other than its vitamin D content. Newlander and Jones (*10*) reported that cows receiving a ration of timothy hay, corn silage, and grain utilized calcium and phosphorus as well as when grass, either green or artificially dried, was added to the ration.

Results reported by Luce (*9*) show that cows receiving a diet of fresh green-pasture grass produced milk of higher antirachitic or growth-promoting properties, even when they were maintained indoors in the absence of direct sunlight, than cows not receiving green grass in the ration. Likewise, Chick and Roscoe (*4*) found some evidence that the inclusion of fresh green feed in the ration caused an increase in the vitamin D content of milk.

It was the purpose of this investigation to measure the comparative calcifying properties of pasture herbage when fed in a green, artificially dried, and sun-cured condition.

EXPERIMENTAL PROCEDURE

The preventive method of procedure used in determining the antirachitic or calcifying properties of the pasture herbage was essentially that outlined by Sherman and Stiebeling (*13*, *14*), in which the basal ration is adequate in other respects but devoid of vitamin D. Young rats 21 to 24 days of age receiving the Sherman and Stiebeling (*13*) vitamin-D-deficient test diet and weighing from 45 to 65 g were used in this investigation. They were placed on experiment when approximately 28 days of age. The animals used were from the California Institute of Experimental Biology, pied strain, reared from mothers whose diet consisted of ground whole wheat, 67.5 percent; casein, 15 percent; whole-milk powder, 10 percent; milk fat, 5 percent; sodium chloride, 1 percent; and calcium carbonate, 1 percent. Fresh lettuce was fed twice each week.

During the experiment the rats were kept in individual, all-metal cages with raised screen floors and maintained in a darkened room devoid of all natural light. The basal diet consisted of cornstarch, 66 percent; extracted casein, 18 percent; dried brewers' yeast, 10 percent; Osborn and Mendel salt mixture, 4 percent; c.p. sodium chloride, 1 percent; and dried spinach, 1 percent. In those instances in which rats were receiving pasture herbage as a part of their ration the spinach, which supplied vitamin A, was eliminated and the percentage of cornstarch correspondingly increased. The rats were allowed free access to their feed at all times, and clean fresh water was always before them. Individual live weights were recorded each week and feed consumption was checked daily.

In comparing the calcifying properties of pasture herbage fed in the different ways, two experiments were conducted. The first test was a comparison of the calcifying properties of green with artificially dried pasture herbage; the second compared artificially dried with sun-cured herbage. In each test negative and positive control groups were maintained. The negative control groups received the basal ration only, whereas the positive control groups received, in addition to the basal ration, 4 drops of 250 D viosterol per week. The viosterol was fed directly into the mouths of the rats.

The material being tested was fed at 3-percent, 6-percent, and 9-percent levels. The percentage of herbage in the experimental rations was calculated on a dry-matter basis.

Insofar as possible the animals were assigned to control and test groups so that litter mates and initial live weight would be equally distributed among the various lots. In the first experiment an equal distribution of sex was maintained in each group. In the later experiment, only male rats were used. It was found necessary for reasons not attributed to this investigation to discontinue the first experiment at the end of the seventh week. Therefore, the mean ash analyses of the first experiment are not on a strictly comparable age basis with those of the second experiment, which terminated at the end of the eighth week. This should not, however, interfere with the interpretation of the results, for a comparison of improvement in the test animals over their respective negative controls, relative to the improvement of the positive over the negative controls, is used as the basis of interpretation.

In outlining the procedure of this method of quantitative determination of vitamin D, Sherman and Stiebeling (*14, pp. 689, 692*) make the following statement:

> It will be noted that our basal diet is radically different from the rickets-producing diets commonly used for vitamin D studies which involve the use of the line test. None of our rats receiving only the basal diet here used developed rickets as judged by the line test, although occasionally a few beaded ribs were observed in rats 80 days or more in age. We are not dealing with rickets as ordinarily understood.
>
> We consider to have equivalent vitamin-D content those amounts of materials under investigation which induce a degree of calcification midway between the minimum values fixed by the diet without added vitamin D and the maximum values obtained with an abundance of supplementary vitamin D. It should be emphasized that this method is feasible only when the groups of animals used for testing vitamin D can be compared with two control groups each containing representatives from the same litters and matched in sex and weight, one receiving no added vitamin D, the other receiving a fixed liberal supply.

At the termination of the experimental periods the rats were chloroformed and the femur bones dissected out, carefully cleaned of all adhering tissue, weighed, dried, extracted with alcohol, reweighed, and ashed. The percentage of ash in the fresh femur bone was used as a criterion of the degree of calcification.

The pasture herbage used in this study was a mixture of English ryegrass (*Lolium perenne*), Italian ryegrass (*L. italicum*), and a small percentage of white clover (*Trifolium repens*). The pasture was irrigated with a sprinkler at weekly intervals to insure uniform rate of growth throughout the season. The samples of herbage used, with the exception of that fed green, were collected once each week, at 1 p.m., and represented herbage that was 3 weeks old.

The 3-weeks-old grass that was fed in a green condition was cut daily at 1 p.m., mixed, and ground into a fine mash. This was thoroughly mixed with the basal ration at the desired percentage for each rat. The resultant mixture was a dry, sticky mash that proved to be quite palatable to the animals.

The samples that were to be artificially dried were taken to the drier immediately after being cut. Drying was accomplished by means of a direct heat rotary drier. The material passed through the machine in about 10 minutes and was dried at an outlet temperature of 165° to 175° C.

The herbage to be sun-cured was exposed on a canvas to the direct rays of the sun and was withheld from any leaching that might have resulted from rain or dew. The exposure to direct sun's rays of varying intensity during the drying process averaged about 15 hours. The exposure of each portion of the sun-dried grass took place over a 2-day period.

In all cases the herbage was thoroughly mixed, finely ground, and remixed prior to its incorporation into the experimental rations. The ration containing as much as 9 percent of grass (dry-matter basis) was readily eaten by the experimental animals.

RESULTS

Table 1 gives a summary of the first experiment and shows the effect upon growth and calcification in rats of adding graded portions of green and artificially dried pasture herbage to the basal diet. Table 1 also summarizes the records of the second test and shows the effect upon growth and calcification of adding graded portions of dehydrated and sun-cured herbage to the basal diet.

In interpreting the antirachitic potency of materials studied by the preventive method of procedure, investigators have measured results in the following ways: (1) Percentage of ash in the green femur bone, (2) percentage of ash in the dried extracted femur bone, and (3) the ratio of the ash to the organic residue of the femur bone.

In this investigation the average of 150 observations of rat bones in which the percentage of ash in the green femur bone was correlated with the percentage of ash in the same bone after it had been dried and extracted revealed a coefficient of 0.880 ± 0.039. The correlation between the percentage of ash in the green femur bone and the ash-organic-residue ratio was 0.743 ± 0.025. These high degrees of correlation illustrate the possibility of using any one of the three methods as a criterion of the degree of calcification in the bones of rats. The method of basing the results upon the percentage of ash in the green femur bone has the advantage of being less time consuming and less expensive.

The data on mean percentage of bone ash and gain in live weight show smaller differences between the negative and positive control groups in the first experiment than in the second. However, in each experiment the range of difference between the negative and positive control group was statistically significant.

In this connection, Sherman and Stiebeling (*13, pp. 500–501*) state:

> Although we have encountered wide seasonal and litter variations in the level of calcification found in both negative and positive controls, we find that this does not interfere with our interpretation of results when we use as a basis of comparison the improvement in test animals over their respective negative controls, relative to the improvement of the positive over the negative controls.

TABLE 1.—*Effect upon growth and calcification of adding graded portions of green, artificially dried, and sun-cured pasture herbage to the basal diet of rats, for experimental periods of 49 and 56 days*

Group	Quantity of pasture herbage (dry-matter basis) added to the basal diet	Cases	Average initial live weight	Average [a] gain in live weight	Average weight of green femur bone	Ash in green femur bone		Improvement over negative controls		
								Per-cent	Critical ratio [a]	Relative to positive controls
FIRST EXPERIMENT (GREEN AND ARTIFICIALLY DRIED GRASS COMPARED), 49 DAYS										
	Percent	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*			*Percent*
Negative controls, fed basal diet only		10	63 0±0 59	140. 0±1. 42	0 5945±0. 081	0 2198±0. 056	35. 60±0 22			
Groups receiving green grass in addition to the basal diet	3	9	63 9± . 53	151 2±1. 65	6115± . 051	. 2400± . 034	38. 78± . 32	3 18	8	82
	6	10	65 0± 67	142 0±1 44	6045± . 081	. 2360± . 060	38. 60± . 23	3 00	9	77
	9	10	64 0± . 48	148. 0±1 22	. 6045± . 045	. 2400± . 047	39. 80± . 36	4 20	10	108
Groups receiving artificially dried grass in addition to the basal diet	3	10	66 0± 47	136 0±1 54	5845± 077	2280± . 039	38 80± . 28	3. 20	9	82
	6	8	65. 0± 65	137. 5±1. 40	6065± . 092	. 2375± . 068	38. 75± . 19	3 15	11	81
	9	10	63 0± 59	142 0±1 24	. 5945± . 054	. 2380± . 044	39. 20± . 28	3 60	10	92
Positive controls, given viosterol in addition to the basal diet		8	63. 8± . 70	145. 0±1. 03	5945± . 051	. 2325± . 037	39. 50± . 28	3. 90	11	100
SECOND EXPERIMENT (ARTIFICIALLY DRIED AND SUN-CURED GRASS COMPARED), 56 DAYS										
Negative controls, fed basal diet only		10	68. 0±1 00	138 0±1 21	5945± 045	. 1860± . 065	30 60± . 43			
Groups receiving artificially dried grass in addition to the basal diet	3	9	65 0± . 67	237 8± 88	. 7555± 022	3022± . 024	39. 12± 26	8. 52	17	89
	6	9	67 2± 44	220 0±1 16	. 7445± 040	2888± . 030	38. 66± 26	8. 06	16	84
	9	9	67 2± 65	240 0± . 95	7445± 071	2956± . 047	40 00± . 30	9. 40	18	98
Groups receiving sun-dried grass in addition to the basal diet	3	10	67 0± 59	226 0±1 28	. 7345± 047	. 2880± . 051	38 40± . 27	7. 80	15	81
	6	10	66 0± . 47	226 0±2. 26	7245± 079	. 2820± . 067	39 00± . 07	8. 40	10	88
	9	9	62. 8± . 45	248 8±2 08	7995± . 059	. 3156± . 058	39. 56± 25	8. 96	18	93
Positive controls, given viosterol in addition to the basal diet		10	66 0± . 77	238 0±1 73	. 7745± . 068	. 3100± . 054	40. 20± . 21	9. 60	20	100

[a] Critical ratio indicates ratio of the difference to its probable error.

Had it been possible to continue the first experiment through the eighth week, the differences noted probably would have been less pronounced. The fact that this experiment was conducted during midsummer may also have been a contributing factor, although the test animals were cared for in a room devoid of sunlight or other natural light.

Animals receiving additions of green, or artificially dried, or sun-cured pasture grass in the basal diet all showed significantly higher calcification than did the negative controls. The mean percentage of bone ash for each group, except those receiving 9 percent of green grass as a supplement, fell within the range of differences existing between the negative and positive controls.

When either green or artificially dried pasture herbage was fed as 3 percent of the dry matter of the ration, there was an improvement in the percentage of bone ash, which amounted to 3.18 percent and 3.20 percent, respectively. In the second test, when either the artificially dried or the sun-cured herbage was fed at the 3-percent level, the increases over the negative controls were 8.52 percent and 7.80 percent, respectively. Further additions of 6 and 9 percent of the various supplements to the diet did not materially increase the degree of calcification. Apparently there was sufficient of the calcifying factor in the green grass, artificially dried grass, or sun-cured grass when fed as 3 percent of the ration to cause good calcification in the experimental animals. Whether this marked improvement in calcium deposition was due entirely to vitamin D, or, as Hart and his coworkers (reported by Clark (*5*)) have suggested, was partly due to other factors contained in green plant tissue, remains unsolved. In any event it is apparent that the potency was relatively the same in the green, the artificially dried, and the sun-cured herbage used in this investigation. At each of the three levels of feeding, the artificially dried herbage was relatively as efficient in furnishing the calcifying factor as was the green material. Exposure of the herbage to high temperature, therefore, had no deleterious effect upon the factor causing calcification in rats.

Samples of pasture herbage that were exposed to 15 hours of direct sunlight were not appreciably more efficient in promoting calcification than was similar herbage that had been artificially dried. Russell (*12*) found alfalfa to be higher in its antirachitic potency when sun-cured than when machine-dried or air-dried in the absence of sunlight. Smith and Briggs (*16*) reported that alfalfa that had been exposed to Arizona sunlight for 15 hours and 5 minutes possessed only mild calcifying properties. In their assays these workers used the line test with a high-calcium, low-phosphorus diet.

According to Bethke, Kick, and Wilder (*2*) the ratio of calcium to phosphorus in the ration produces a marked effect upon the growth and calcification in rats. They state that the most favorable calcium-phosphorus ratio for growth and bone development was between 2.00 and 1.00 of calcium to 1.00 of phosphorus, and that the vitamin D requirements were higher when the ratio was widened beyond these limits. Table 2 gives the percentage of calcium and phosphorus and the calcium-phosphorus ratio for each of the rations used in the second experiment of this investigation. It will be noted that these rations contained considerably more phosphorus, and therefore probably had a more favorable ratio, than the diets used by

Russell (*12*) or by Smith and Briggs (*16*). The calcium-phosphorus ratios of the experimental rations used in this investigation differed so little that differences in the degree of calcification could not be attributed to differences in this ratio.

TABLE 2.—*The calcium and phosphorus content and the ratios of calcium to phosphorus in the rations of the second experiment*

Ration	Calcium	Phosphorus	Ca/P
	Percent	*Percent*	
Basal diet	0.684	0.705	0.97
Basal mixture with the addition of—			
3 percent of artificially dried grass	.668	.705	.95
6 percent of artificially dried grass	.663	.680	98
9 percent of artificially dried grass	.663	.659	1.00
3 percent of sun-dried grass	.634	.692	.92
6 percent of sun-dried grass	.666	.673	.99
9 percent of sun-dried grass	660	665	99

Evidence is herein presented which confirms the belief that when the calcium and phosphorus intake of the animal is approximately normal, green plant tissue is a contributing factor in causing calcium assimilation in the animal's body. It also appears that this property of green plant tissue is not destroyed by artificial dehydration as employed in this investigation.

CONCLUSIONS

In this paper data are presented to show the comparative calcifying powers of similar samples of green, artificially dried, and sun-cured pasture herbage. These materials were incorporated in the rations at the rate of 3, 6, and 9 percent of the total dry matter.

Pasture herbage when fed in a green, artificially dried, or sun-cured condition, constituting 3 percent of the dry matter in the ration, caused a significantly greater degree of calcification in rats than did the basal diet.

Increasing the amount of the different types of herbage fed failed to produce corresponding increases in the degree of calcium deposition.

The process of dehydration by subjecting the material to high temperatures for a short period, did not destroy the potency of the calcifying property of the herbage. Either the green or the artificially dried grass was as efficient in producing calcification as was similar herbage cured by exposure to 15 hours of sunlight.

A correlation coefficient of 0.880 ± 0.039 was found to exist between the percentage of ash in the green femur bone and that of the dry extracted bone. The correlation coefficient between the percentage of ash in the fresh femur bone and the ratio of the ash to the organic residue in the femur bone was 0.743 ± 0.025.

The result of this investigation furnished further evidence of the high nutritive value of pasture herbage.

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INJURY FROM CALCIUM ARSENATE-HYDRATED LIME SPRAY ON SNAP BEANS RETARDED IN GROWTH BY UNFAVORABLE SOIL CONDITIONS[1]

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INTRODUCTION

Observations made on commercial acreages of beans following treatments with arsenicals occasionally indicated arsenical injury in scattered sections of a field without apparent injury to the field as a whole. It appeared that a retardation in the growth of the plants associated with an unfavorable soil reaction might account for the apparent variation in susceptibility to injury. It was with this in mind that the investigation reported herein was conducted.

REVIEW OF LITERATURE

Although there is frequent mention in the literature of injury to foliage by calcium arsenate, no records have been found of previous investigations on the relation of the hydrogen-ion concentration of the soil and plant growth to the susceptibility of plants to injury from this material. Zimmerley[4] found that the range of hydrogen-ion concentration for the optimum growth of snap beans was from pH 5.3 to pH 6.0; and that the beans were chlorotic, made poor growth, and gave the lowest yields when the naturally acid soils of the Norfolk area were brought to neutral or were made slightly alkaline by the application of hydrated lime.

PROCEDURE

The experiment was conducted on the Bountiful variety of snap beans (*Phaseolus vulgaris* L.) growing in the acidity test plots of the Virginia Truck Experiment Station, a diagram of which is shown in figure 1. Treatments were applied to plots having the following approximate pH values: 7.6, 7.0, 6.5, 6.0, 5.7, 5.2, and 4.8.[5] These plots had been maintained at approximately the foregoing pH values since 1926. The plots at pH 6.5, 6.0, 5.7, 5.2, and 4.8 contained 2 check and 2 treated rows and were duplicated in the field. Those at pH 7.6 and 7.0 contained 1 check and 1 treated row and were replicated four times. Thus there were 4 treated and 4 check rows for each pH value. Grass ridges separated the pH sections, and alleys separated the plots of each section (fig. 1).

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[1] Received for publication Nov. 17, 1933; issued May 1934.

[2] The writer wishes to express his appreciation to members of the staff of the Virginia Truck Experiment Station, and to Neale F. Howard, of the Bureau of Entomology, for suggestions and criticisms in the preparation of the manuscript. During the summer of 1931 the writer was assisted in the experimental work by J. R. Weedon, field assistant.

[3] In cooperation with the Virginia Truck Experiment Station.

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[5] The quinhydrone electrode was used in making the pH determinations. These determinations were made by workers of the horticultural department of the Virginia Truck Experiment Station.

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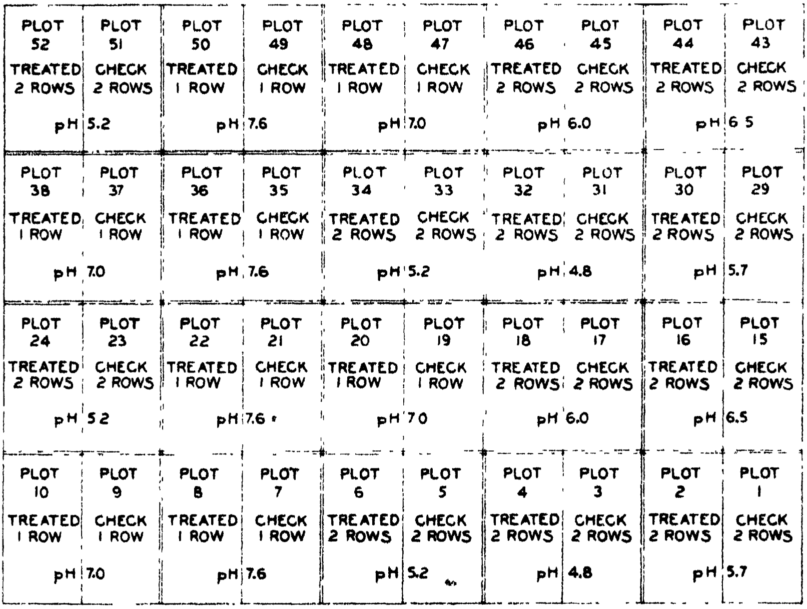
A spray consisting of 1 pound of calcium arsenate and 2 pounds of hydrated lime in 50 gallons of water was selected for the treatments because in previous experimental work this spray had frequently caused arsenical injury to beans in this locality. Treatments were applied on September 1, 8, 15, and 21. Each treatment was applied between 1 p.m. and 5 p.m. At the time of the first treatment the beans had just begun to send out their first trifoliate leaves. The fourth treatment was applied when the plants were blooming and a few pods had formed.

A 2½-gallon compressed-air sprayer was used in applying all treatments, and the sprayer was pumped with the same number of strokes on each plot in order to maintain a uniform pressure. The insecticide was applied at the rate of 100 gallons per acre, and the beans were treated from each side of the row to insure thorough coverage of all parts of the plants.



PLOT 52 TREATED 2 ROWS	PLOT 51 CHECK 2 ROWS	PLOT 50 TREATED 1 ROW	PLOT 49 CHECK 1 ROW	PLOT 48 TREATED 1 ROW	PLOT 47 CHECK 1 ROW	PLOT 46 TREATED 2 ROWS	PLOT 45 CHECK 2 ROWS	PLOT 44 TREATED 2 ROWS	PLOT 43 CHECK 2 ROWS
pH	5.2	pH	7.6	pH	7.0	pH	6.0	pH	6 5
PLOT 38 TREATED 1 ROW	PLOT 37 CHECK 1 ROW	PLOT 36 TREATED 1 ROW	PLOT 35 CHECK 1 ROW	PLOT 34 TREATED 2 ROWS	PLOT 33 CHECK 2 ROWS	PLOT 32 TREATED 2 ROWS	PLOT 31 CHECK 2 ROWS	PLOT 30 TREATED 2 ROWS	PLOT 29 CHECK 2 ROWS
pH	7.0	pH	7.6	pH	5.2	pH	4.8	pH	5.7
PLOT 24 TREATED 2 ROWS	PLOT 23 CHECK 2 ROWS	PLOT 22 TREATED 1 ROW	PLOT 21 CHECK 1 ROW	PLOT 20 TREATED 1 ROW	PLOT 19 CHECK 1 ROW	PLOT 18 TREATED 2 ROWS	PLOT 17 CHECK 2 ROWS	PLOT 16 TREATED 2 ROWS	PLOT 15 CHECK 2 ROWS
pH	5 2	pH	7.6	pH	7 0	pH	6.0	pH	6.5
PLOT 10 TREATED 1 ROW	PLOT 9 CHECK 1 ROW	PLOT 8 TREATED 1 ROW	PLOT 7 CHECK 1 ROW	PLOT 6 TREATED 2 ROWS	PLOT 5 CHECK 2 ROWS	PLOT 4 TREATED 2 ROWS	PLOT 3 CHECK 2 ROWS	PLOT 2 TREATED 2 ROWS	PLOT 1 CHECK 2 ROWS
pH	7.0	pH	7.6	pH	5.2	pH	4.8	pH	5.7

FIGURE 1.—Diagram of the experimental plots and arrangement of the pH sections.

Only a few adults of the Mexican bean beetle appeared in the plots. Injury by this insect was not a factor in the results.

EXPERIMENTAL RESULTS

The results of the experiment are presented in table 1. This table shows the yields of two pickings of beans on the check and treated plots of each pH value. Since the plants in each plot were counted, the percentage increase or decrease, check-plot basis, was calculated from the mean yields in grams per plant. On this basis the treatment resulted in serious reductions in yield on the plots with pH values of 7.6 and 7.0, slight reductions on plots at 6.0 and 5.2, and slight increases on plots at 6.5, 5.7, and 4.8.

TABLE 1.—*Comparative yields of snap beans, treated with an arsenical spray and untreated, grown on plots having different hydrogen-ion concentrations*

pH of soil	Plot no.		Plants per plot		Average yield per plant		Increase (+) or decrease (−) in average yield of treated plants		Estimate of injury of—	
	Check	Treated	Check	Treated	Check	Treated			Check[a]	Treated
			Number	*Number*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*
7.6	7		145		16.6				20	
		8		130		6.1	−10.5			[b]60
	21		140		13.0				20	
		22		156		3.6	−9.4			[b]70
	35		139		12.0				25	
		36		124		1.8	−10.2			[b]80
	49		102		16.9				15	
		50		129		4.8	−12.1			[b]60
Average					14.6	4.1	−10.5	−72		
7.0	9		169		16.1				15	
		10		201		11.0	−5.1			[b]50
	19		177		8.5				20	
		20		124		4.6	−3.9			[b]70
	37		149		17.1				15	
		38		163		13.7	−3.4			[b]55
	47		157		11.9				15	
		48		145		7.6	−4.3			[b]65
Average					13.4	9.2	−4.2	−31		
6.5	15		378		25.6					
		16		358		27.2	+1.6			[c]10
	43		252		26.4					
		44		319		27.0	+.6			[c]10
Average					26.0	27.1	+1.1	+4		
6.0	17		397		31.8					
		18		383		27.7	−4.1			[c]10
	45		336		30.5					
		46		375		26.9	−3.6			[c]10
Average					31.1	27.3	−3.8	−12		
5.7	1		385		28.0					
		2		368		28.1	+.1			[c]10
	29		318		21.03					
		30		335		21.07	+.04			[c]10
Average					24.51	24.58	+.07	+.3		
5.2	5		326		33.5					
		6		318		29.7	−3.8			[c]15
	33		275		28.4					
		34		296		29.3	+.9			[c]10
Average					30.9	29.5	−1.4	−5		
4.8	3		418		22					
		4		441		20.7	−1.3			[c]5–10
	31		375		14.3					
		32		375		20.3	+6.0			[c]5
Average					18.2	20.5	+2.3	+13		

[a] Estimate of chlorosis on plants.
[b] Estimate of arsenical injury and chlorosis
[c] Estimate of arsenical injury alone.

Since the check and treated plots were paired, a biometrical analysis of the data was made by Student's method of comparing two results on a probable-error basis.[6] Odds greater than 30 to 1 that the reductions were not due to chance alone were obtained on the pH 7.6, 7.0, and 6.0 plots. In the case of the first two plots, on which the reductions in yield were 72 and 31 percent, respectively, the odds were very great, approximately 5,000 to 1 and 1,300 to 1, respectively. In the case of the pH 6.0 plot, on which the reduction was 12 percent, the odds of a significant reduction in the yield of the treated plots under the check were only 46 to 1.

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[6] HAYES, H. K., and GARBER, R. I. BREEDING CROP PLANTS. Pp. 86–89. New York. 1927.

The comparative yields in grams per plant on the check and treated plots are shown in figure 2. The maximum check-plot yield was obtained on the pH 6.0 plots, and the optimum range was between pH 6.5 and 5.2.

At the conclusion of the experiment arsenical injury was slight on the pH 6.5, 6.0, 5.7, 5.2, and 4.8 plots, but was estimated at 60 to 80 percent on the pH 7.6 plots and from 50 to 70 percent on the pH 7.0 plots; thus injury was considerably more severe on the plants grown in soil at pH 7.6 and pH 7.0 than on plants grown in soil at any of the other reactions (table 1). The treated plots with pH values of 7.6 and 7.0 showed severe leaf burn, leaf shedding, and stunting.

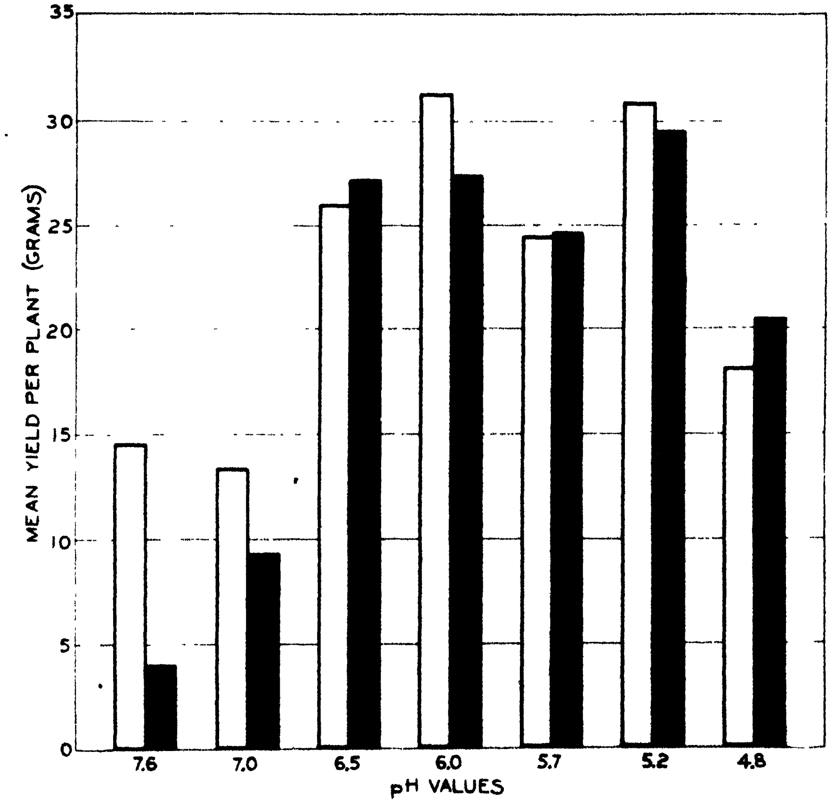

FIGURE 2.—Comparative yields of check and treated plots having different pH values. The unshaded columns represent check plots; the shaded, treated plots.

The arsenical injury to beans on the pH 6.5 to 5.2 plots, which produced the heaviest yields (fig. 2), was not nearly so severe as has frequently been observed on beans grown under the best conditions in this locality. This is probably due to the fact that the relative humidity was comparatively low during the period of the treatments. Previous experience has shown that high humidity increases plant injury from calcium arsenate.

Serious reductions in yield occurred only on the plants grown at pH 7.6 and pH 7.0. On these plots the plants were very chlorotic on both check and treated plots, and the check yields were considerably lower than at any other pH value.

The check yield (fig. 2) from plots with a reaction of pH 6.0 was more than twice as great as the check yields from plots with a reaction of pH 7.6 and 7.0.

The results indicate that snap beans grown under the conditions of this experiment in soils of pH 7.6 and 7.0 are considerably more susceptible to injury from calcium arsenate-hydrated lime spray than are plants grown in soils of pH 6.5 to pH 4.8.

SUMMARY

Four treatments of calcium arsenate-hydrated lime spray (1:2:50) were applied to snap beans grown on soils having approximately the following pH values: 7.6, 7.0, 6.5, 6.0, 5.7, 5.2, and 4.8.

Yields were recorded, and the effect of spraying on yield, check-plot basis, was calculated from the mean yields in grams per plant. On this basis treatment was found to result in serious reductions in per plant yields on the plots with pH values of 7.6 and 7.0, slight reductions on the plots at pH 6.0 and 5.2, and slight increases on the plots at pH 6.5, 5.7, and 4.8.

A biometrical analysis of the data was made by Student's method. Odds greater than 30 to 1 that the reductions were not due to chance alone were obtained on the pH 7.6, 7.0, and 6.0 plots. In the case of the first two plots, on which reductions in yield were 72 and 31 percent, respectively, the odds were very great, approximately 5,000 to 1 and 1,300 to 1, respectively. In the case of the pH 6.0 plots, on which the reduction was 12 percent, the odds of a significant reduction in the yield of the treated plots under the check were only 46 to 1. Arsenical injury was considerably more severe on the plants grown in soil at pH 7.6 and pH 7.0 than on plants grown in soil at any of the other reactions. Severe chlorosis was apparent on check and treated plots, and check yields were considerably lower than at any other pH value.

The maximum check-plot yield was obtained on the pH 6.0 plots; the optimum range was between pH 6.5 and pH 5.2.

The results of the experiment indicate that snap beans retarded in growth by unfavorable soil conditions are decidedly more susceptible to arsenical injury from calcium arsenate-hydrated lime spray than are plants grown under optimum conditions.

THE EFFECT OF HYPERTONIC SUGAR SOLUTIONS ON THE THERMAL RESISTANCE OF BACTERIA[1]

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INTRODUCTION

In connection with the various studies on the bacteriology of ice cream which have been in progress at the Kansas Agricultural Experiment Station for several years, it has been observed occasionally that micro-organisms exhibit an increased thermal resistance when heated in ice-cream mix. Preliminary experiments showed quite definitely that certain strains of bacteria were capable of withstanding more severe heat treatment when suspended in solutions of high osmotic pressure.

The temperature and time of exposure most commonly employed in the pasteurization of ice-cream mix have been adopted from the market-milk industry without question as to the universality of their application. If thermal resistance is affected by the chemical and physical forces of the environment it is entirely logical to expect a variation in the survival of cells heated in different menstrua. The small margin of safety in the present requirements for pasteurization emphasizes the importance of evaluating any increase in thermal resistance of the microflora which may be contributed by the ingredients of ice cream.

REVIEW OF LITERATURE

Until comparatively recently the protective action of the ingredients of ice-cream mix for micro-organisms has not been recognized. Beavens (*4*)[2] found that 4- to 20-percent lactose increased the thermal resistance of *Escherichia coli*. The probability that the ingredients of ice cream might afford micro-organisms some protection against heat was recognized by the Committee on Dairy Products and Eggs (*15*). The results of Oldenbusch, Frobisher, and Shrader (*8*) gave only slight evidence of increased survival of various pathogenes when heated in ice-cream mix or in cream containing 50 percent fat. Fabian and Coulter (*6*) observed higher thermal death points for cultures of *E. coli* and *Aerobacter aerogenes* when heated in ice-cream mix than when heated in skim milk. Except for sucrose, these authors were unable to show any marked protective effect when the ingredients of ice cream were studied separately.

Anzulovic (*1*) reported that sugar, gelatin, serum solids, and fat showed some protective action for bacteria. Weiss (*17*) found that *Bacillus botulinus* was more resistant to heat in foods containing heavy sirups. Rahn (*9*) stated that—

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[1] Received for publication Oct. 17, 1933; issued May 1934. A résumé of a dissertation submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy. The work was made possible through the cooperation of the Department of Dairy Industry, Iowa State College of Agriculture and Mechanic Arts, and the Department of Bacteriology, Kansas State College of Agriculture and Applied Science. Published as doctoral dissertation no. 244, Iowa State College, and contribution no. 154 of the Department of Bacteriology, Kansas State College.

[2] Reference is made by number (italic) to Literature Cited, p. 467

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> * * * sugar not only retards growth of yeasts and other micro-organisms, but also retards the action of heat upon micro-organisms; it will take more heat to kill a bacterium or yeast in a sweetened fruit juice than in the same juice without sugar.

Robertson (*11, 12, 13, 14*) heated *Streptococcus thermophilus, Sarcina lutea, Escherichia coli,* and *Micrococcus aureus* in increasing percentages of sugar and found that as the concentration of sugar was increased the number of surviving bacteria also increased.

Nechkovitch (*7*) showed that glucose tended to prevent the coagulation of cell colloids and aided in maintaining a normal condition of stability of the cells of organized tissue. Wallace and Tanner (*16*) suspended several species of molds in 10-, 25-, and 50-percent sugar, distilled water, sirups from fruit juices, and in salt water. Protective action was afforded by sugar for some molds and by salt water for others.

Rahn (*10, p. 330*) reported increased thermal resistance of yeasts and bacteria when heated in broth containing 50 percent of sucrose. This author suggested that although death may be due in part to dehydration, the cause of death is not the same as with dry bacteria. Cook[3] explained his observations of increased resistance of yeasts in hyper tonic glucose and sucrose solutions on the basis of dehydration of the cells. He stated, however, that there was probably some factor other than osmotic pressure involved, since the killing times were not proportional to the osmotic pressure of the solutions employed.

Beilinsson (*5*) observed that the addition of sufficient sucrose or glycerol to serum albumin or to egg white rendered these proteins stable to temperatures far above the usual coagulation points. Bancroft and Rutzler (*3*) and Bancroft and Richter (*2*) confirmed these observations and suggested an explanation based upon the peptizing action of sugar on albumin. According to these authors, if the colloidal suspension is reversibly coagulated, the cell may have lost temporarily any or all of its vital manifestations, but will recover from dormancy when placed in a favorable environment. As agglomeration of the cell contents increases, the cell loses more and more of its functions, the coagulation becomes progressively less reversible and finally completely irreversible.

METHODS

In all the plating procedures standard beef-extract agar was employed as the basic medium. In those instances in which comparisons were made between the counts on plain and carbohydrate media, a large batch of the plain agar was divided into equal parts and 1 percent of the desired carbohydrate added to each portion. The reaction of all media was adjusted to pH 7.0 before filtration.

The distilled water used in making dilution blanks occasionally was tested after sterilization for its reaction and found to be pH 6.0 ± 0.2.

All plates were incubated 48 hours at 37° C. In several of the experiments where delayed germination was suspected, counts were made again after an additional 3 days' incubation at room temperature.

In several experiments reference is made to the use of a 100-percent sugar solution. This refers to a weight-volume ratio, and the solution was prepared in the following manner: Approximately 250 cc of water was added to 1 kg of the desired sugar, and this was boiled for a few minutes until a clear solution was obtained. The resulting sirup (approximately 900 cc) was diluted to 1,000 cc total volume. Such a solution therefore contained 1,000 g of sugar in a total volume of 1,000 cc, and each cubic centimeter represented 1 g of sugar. All the other sugar solutions employed, the concentrations of which are expressed in percentages, were prepared by suitable dilution of this 100-percent sugar solution. The sugar solutions, the concentrations of which are expressed in molality, were prepared by dissolving the indicated number of gram-molecular weights in 1,000 cc of water.

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[3] COOK, W. B. STUDIES ON THE STERILIZATION OF SOLUTIONS OF GLUCOSE AND SUCROSE. (Thesis, Ph.D., Iowa State College.) 1931.

In order to reduce the factor of heat penetration to a practical minimum, small samples were used in all heating trials. In some cases, 1.5 to 2.0 cc samples were placed in small hermetically sealed tubes and completely submerged in a water or an oil bath for the desired heating period. In other cases, thin-walled, small-bore (4 mm) test tubes were submerged in the oil bath to within 1 inch of the top of the tube. For many of the experiments special tubes were prepared by blowing a bulb about 1.5 inches in diameter on the end of a soft glass test tube. The small sample in the relatively large bulb of very thin glass acquired the temperature of the water bath very quickly. Obviously, heat penetration would be delayed in samples containing high percentages of sugar. However, the importance of this factor was shown to be reduced to a negligible minimum when small samples (2 cc or less) were employed. Graphs illustrating the rates of heat penetration under the conditions of these experiments show practically superimposed lines.

Although the results of the preliminary experiments did not indicate that it was necessary, all tubes containing more than a 0.1 cc sample were agitated by uniform shaking during the heating period.

RESULTS

HYPERTONIC SOLUTIONS IN ICE CREAM MIX

A sample of ice cream mix which contained no sucrose was incubated at room temperature until the bacterial count reached several million per cubic centimeter. This was divided into four parts and equal volumes of sterile sucrose solutions were added to give 15-, 25-, and 50-percent concentrations of sugar. A similar volume of water was added to one portion, which was designated as 0-percent sucrose. Plates were made before and after heating small portions of the four samples simultaneously to 54.5° C. for 9 minutes. In order to facilitate comparison, the number of viable organisms after heating has been calculated on the basis of survival per million.

The data in table 1 were compiled from the results of four such trials, and illustrate the general trend of many similar experiments. It will be observed in experiment 1 that the ice cream mix had a plain agar plate count of 14,000,000 per cubic centimeter before heating. After heating, the survival per million was 450 in the sample containing no sucrose, and 35,000, 44,000, and 35,000 in the samples containing 15-, 25-, and 50-percent concentrations of sucrose, respectively. Since all four samples were identical except for the sugar, the results suggest that the presence of sugar in ice cream renders the cells more resistant to heat.

TABLE 1.—*The effect of plain and carbohydrate agars on the survival of micro-organisms in ice cream mix containing various amounts of sugar after heating at 54.5° C. for 9 minutes*

Experiment no	Agar	Count per cubic centimeter before heating	Survival per million after heating in ice cream mix containing—			
			0 percent sucrose	15 percent sucrose	25 percent sucrose	50 percent sucrose
		Millions	*Number*	*Number*	*Number*	*Number*
1	Plain	14	450	35,000	44,000	35,000
2	Plain	170	310	1,500	2,800	25,000
	Dextrose	180	19,000	14,000	14,000	61,000
	Sucrose	130	24,000	18,000	22,000	77,000
	Lactose	170	19,000	18,000	22,000	59,000
3	Plain	110	220	5,500	5,900	44,000
	Sucrose	110	5,500	8,000	8,000	39,000
4	Plain	66	260	7,900	7,900	18,000
	Sucrose	60	17,000	18,000	17,000	18,000

In the other experiments reported in table 1 the same routine was followed except that various carbohydrate agars were employed in pouring parallel plates from dilution blanks. In experiment 2, for example, the survival per million in the sample containing no sucrose was 310 when plated on plain agar, and from 19,000 to 24,000 on carbohydrate agars. This at once suggests that some of the cells, injured but not irreparably destroyed by the heat, were capable of recovery when the medium was fortified with a carbohydrate. In the samples containing 15- and 25-percent sucrose the plain agar counts per million were 1,500 and 2,800, respectively, which indicates that the presence of sugar in the sample diminished the degree of injury to the cells and enabled more of them to recover even in plain agar. Transfers from the same dilution blank to carbohydrate media gave survival per million values ranging from 14,000 to 22,000, again emphasizing the importance of the medium in the recovery of injured cells.

The protective action of sugar is most effectively illustrated in the samples containing 50 percent of sucrose. The thermal resistance of the cells was greatly increased in the presence of such excessive amounts of sucrose.

A consideration of the results of experiments 3 and 4 leads to the same general observations, viz, in ice-cream mix without sugar injury of the cells by heat is greater than when sugar is present; the number of organisms capable of surviving increases with increasing percentages of sugar in the mix (this is especially noticeable when 50 percent of sugar is employed); and more of the injured cells survive in carbohydrate media.

If high osmotic pressures result in an increased thermal resistance for cells, it is conceivable that micro-organisms whose normal resistance is at the threshold of the thermal exposure of pasteurization may survive in ice cream and not in milk.

HYPERTONIC SOLUTIONS IN MILK

Sterile milk was heavily inoculated with a pure culture of *Escherichia coli* 57 and then diluted (1) with an equal volume of water, and (2) with an equal volume of 100-percent sucrose, thereby giving an ultimate concentration of 50-percent sugar. These two samples were plated before and after heating (54.5° C., 9 minutes) on plain agar and on 1-percent dextrose, sucrose, and lactose agars.

The results in table 2 show that approximately 5 to 10 times as many organisms survived in the milk to which sucrose was added. In this experiment the use of carbohydrate agar did not increase the number of survivors—an observation that tends to discourage the conclusion that the injured cells are necessarily rendered more saccharophilic.

TABLE 2.—*Effect of various media on the recovery of Escherichia coli 57 heated at 54.5° C. for 9 minutes in suspensions of milk and milk containing 0- and 50-percent*

Medium	Count per cubic centimeter before heating		Survival per million after heating at 54.5° C. for 9 minutes	
	0-percent sucrose	50-percent sucrose	0-percent sucrose	50-percent sucrose
Plain	10,000,000	10,000,000	48,000	250,000
Dextrose	14,000,000	11,000,000	20,000	230,000
Sucrose	10,000,000	11,000,000	18,000	245,000
Lactose	12,000,000	15,000,000	30,000	150,000

HYPERTONIC SOLUTIONS IN BROTH

PROTECTIVE ACTION OF VARIOUS SUGARS FOR ESCHERICHIA COLI HELD FOR VARIOUS LENGTHS OF TIME

Uniform suspensions of *Escherichia coli* 25 were prepared in plain broth and in broths containing 50 percent, respectively, of maltose, sucrose, and dextrose. Because of the low solubility of lactose, suspensions of cells in saturated lactose broth were prepared separately. These suspensions were plated as quickly as possible on plain agar before and after heating to 54° C. for 9 minutes. After the original suspensions had stood at room temperature for 2 hours, plates were again made before and after heating to 54° for 9 minutes.

Table 3 shows that saturated lactose failed to protect *Escherichia coli* 25, and that 50-percent maltose broth afforded only very slight protection. The survival per million values in 50-percent sucrose and in 50-percent dextrose broths were increased ninefold and sevenfold, respectively, after the cultures had aged 2 hours.

TABLE 3.—*Thermal resistance of Escherichia coli 25 after 2 hours' contact with plain broth, saturated lactose broth, and 50-percent maltose, sucrose, and dextrose broths*

Cell suspension	Count per cubic centimeter after—				Survival per million after—	
	0 hour contact		2 hours contact			
	Before heating	After heating at 54° C. for 9 minutes	Before heating	After heating at 54° C. for 9 minutes	0 hour contact	2 hours contact
Plain broth	500,000	50	550,000	730	100	1,330
Saturated lactose broth	500,000	30	520,000	0	60	0
50-percent maltose broth	500,000	1,100	130,000	380	2,200	2,900
50-percent sucrose broth	340,000	9,800	220,000	58,000	29,000	264,000
50-percent dextrose broth	600,000	18,000	60,000	13,000	30,000	217,000

EFFECT OF PROLONGED EXPOSURE OF CELLS TO HYPERTONIC SUCROSE SOLUTION

Suspensions of *Escherichia coli* 52 in plain and 50-percent sucrose broths were held at 30° C. Samples were removed from the sucrose suspension after each 15-minute interval for 2 hours and then at less frequent intervals for 7 hours. Each sample removed was plated on plain agar before and after heating at 54.5° for 5 minutes.

A study of the results presented in table 4 shows a marked increase in the thermal resistance during the first 2 hours. It is significant to note that although the counts on the sucrose broth before heating were fairly constant, the actual number of cells capable of surviving the heat treatment increased. Evidently the physical changes responsible for greater heat stability of the protoplasm affect an increasing number of cells with time. In this instance the maximum number of cells capable of withstanding the heat treatment occurred after 2 hours' exposure, whereas continued contact with the sugar resulted in a decrease in the thermal resistance.

TABLE 4.—*The thermal resistance of Escherichia coli 52 after prolonged incubation at 30° C. in plain and in 50-percent sucrose broth before heating at 54.5° C. for 5 minutes*

Period of contact before heating (minutes)	Count per cubic centimeters in—				Survival per million in	
	Plain broth		50-percent sucrose broth			
	Before heating	After heating at 54.5° C. for 5 minutes	Before heating	After heating at 54.5° C. for 5 minutes	Plain broth	50-percent sucrose broth
0	1,500,000	10	1,700,000	42,000	7	25,000
15			1,700,000	170,000		100,000
30			1,800,000	450,000		250,000
45			2,200,000	570,000		259,000
60	2,600,000	0	2,300,000	750,000	0	326,000
75			1,700,000	870,000		512,000
90			1,800,000	960,000		533,000
105			1,700,000	690,000		406,000
120	4,100,000	0	1,400,000	1,100,000	0	786,000
180	38,000,000	0	2,900,000	550,000	0	190,000
240	150,000,000	0	1,200,000	350,000	0	292,000
300	220,000,000	10	1,100,000	210,000	0	191,000
420	330,000,000	0	930,000	57,000	0	61,000

The values for the survival per million in the 50-percent sucrose broth have been plotted against time in figure 1. The graph clearly shows the tendency for the cells to become progressively more resistant to heat when exposed to hypertonic sucrose solutions for 2 hours and the tendency to lose this faculty on continued exposure.

These results suggest that high concentrations of sucrose may induce a physical change which no doubt is regulated by the permeability of the individual cell. This process, although ultimately leading to the death of the cell, at first increases the stability of the protoplasm to heat. Continued exposure, however, advances the degree of coagulation to a point beyond which plain agar can no longer induce peptization. The ultimate decline in the number of cells capable of surviving the heat treatment apparently is the result of prolonged desiccation beyond the point of optimum stability.

HYPERTONIC SOLUTIONS IN WATER

Effect of Variations on Osmotic Pressure

Escherichia coli 52 was suspended directly in hypertonic solutions of dextrose and sucrose of various molalities. One tenth cubic centimeter of an 18-hour plain-broth culture was placed in 5-cc quantities of the various sugar solutions; after thorough agitation these were allowed to stand at room temperature for 5 minutes. Two cubic centimeter portions of each suspension were then heated to 55° C. for 5 minutes. Plate counts were made on plain and dextrose agars before and after heating. The data, calculated to a basis of survival per million, are presented in table 5. Owing to the fact that each solution was inoculated separately, there was some unavoidable variation in the initial number of organisms in the solutions

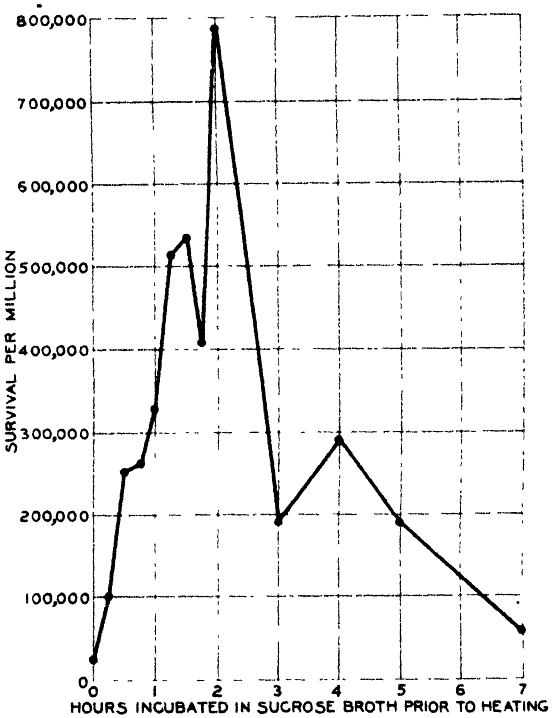

FIGURE 1.—Thermal resistance of *Escherichia coli* 52 after prolonged incubation at 30° C. in 50-precent sucrose broth before heating at 54.5° C. for 5 minutes.

TABLE 5.—*Survival per million of Escherichia coli 52 when heated in equimolal dextrose and sucrose solutions*

Molality of sugar solutions	Survival per million of *E. coli* when heated in—				Molality of sugar solutions	Survival per million of *E. coli* when heated in—			
	Dextrose solutions		Sucrose solutions			Dextrose solutions		Sucrose solutions	
	Plain agar	Dextrose agar	Plain agar	Dextrose agar		Plain agar	Dextrose agar	Plain agar	Dextrose agar
0.25	0	0	180	0	1.5	4,700	12,000	170,000	210,000
0.50	0	450	0	180	2.0	15,000	15,000	360,000	330,000
0.75	0	600	270	21,000	3.0	47,000	31,000	270,000	320,000
1.0	90	360	11,000	100,000	4.0	150,000	100,000	150,000	190,000

Since the osmotic pressures of the equimolal solutions were identical the differences observed in the protective action cannot be accounted for on this basis. It is possible, however, that there were differences in the effective osmotic pressure at the cell surfaces, since this would depend upon the permeability of the individual cell membrane. These results are in harmony with those of Cook[4] to which reference has already been made.

It is evident from table 5 that very little protective action is afforded by either sugar in concentrations below 1 molal. The values in table 5 show quite forcibly that there is an increased protective action with the increasing osmotic pressure beyond 1-molal concentrations.

EFFECT OF ADDING SUGAR AFTER HEATING

In order to determine whether the increased survival of cells heated in hypertonic sugar solutions was attributable to the sugar carried over from the sample into the medium, the following experiment was performed. A 24-hour plain broth culture of *Escherichia coli* 52 was diluted with an equal volume of water in one tube, and with an equal volume of 100-percent sucrose solution in another. These cultures were heated to 54.5° C. for 5 minutes. After heating, the water suspension was divided into two equal parts; an equal volume of 100-percent sucrose solution was added to one part, and the other was similarly diluted with water. This provided (1) cells which had been heated in water and then the sugar added (50-percent concentration) after heating, and (2) cells heated in water and subsequently further diluted with water. In a similar manner the original suspension of cells which had been heated in 50-percent sucrose solution was divided into two parts, one of which was diluted with an equal volume of 50-percent sucrose solution and the other with an equal volume of water. If cells suspended in sucrose surround themselves with a layer of sugar which ultimately serves as an intimate source of energy in the agar plate, one would expect the suspension to which sugar was added after heating to exhibit approximately the same survival as the cells heated in the presence of sugar.

TABLE 6.—*Effect of adding 50-percent sucrose to cell suspensions of Escherichia coli 52 after heating at 54.5° C. for 5 minutes*

[Controls suspended in water]

Treatment of cells	Agar	Count per cubic centimeter		
		Before heating	After heating at 54.5° C. for 5 minutes [a]	Survival per million
Heated in 50-percent sucrose, then equal volume of 50-percent sucrose added	Plain	78,000,000	2,600,000	67,000
	Dextrose	80,000,000	6,700,000	168,000
Heated in water, then equal volume of 50-percent sucrose added	Plain	190,000,000	300,000	3,200
	Dextrose	230,000,000	260,000	2,300
Heated in water, then equal volume of water added.	Plain	190,000,000	2,100,000	22,000
	Dextrose	230,000,000	1,100,000	10,000

[a] Since the samples were diluted with equal volumes of sucrose solution or water after heating, the values in this column should be doubled. The values for survival per million have been adjusted accordingly.

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[4] COOK, W. B. See footnote 3.

The results in table 6 show that the addition of sugar after the cells had been heated in water failed to induce recovery from injury; in fact, the values for survival per million were actually smaller than those observed in the water suspension. The damage to the cells heated in water apparently cannot be counteracted by the subsequent addition of sugar. If the sugar is present during the heating a relatively large number of the cells are protected from irreparable injury.

EFFECT OF HYPERTONIC SUGAR SOLUTIONS ON THERMAL RESISTANCE OF VARIOUS BACTERIA

Some of the results already presented suggest that the permeability of the individual cell membrane may play an important part in the phenomenon of protective action. The results of several experiments not presented in this paper have suggested further that the protective action of dextrose and sucrose solutions was best demonstrated with organisms which were sensitive to heating in water. Considering these individual variations one would not expect, therefore, that all bacteria would be protected by hypertonic solutions.

The protective action of dextrose and maltose was tested on pure cultures of several different organisms. One cubic centimer of an 18-hour plain broth culture of the test organism was inoculated into each of three flasks containing 40 cc of water, 2-molal dextrose, and 2-molal sucrose, respectively. After thorough agitation samples were removed for plating and heating. The time of heating was 5 minutes in all cases, but the temperature employed varied with the different organisms.

TABLE 7.—*Effect of hypertonic solutions on the thermal resistance of cultures of several different organisms*

Culture	Count per cubic centimeter before heating in			Temperature of exposure for 5 minutes	Count per cubic centimeter after heating in		
	Distilled water	2-molal dextrose	2-molal sucrose		Distilled water	2-molal dextrose	2-molal sucrose
				°C			
Serratia marcescens	28,000,000	23,000,000	17,000,000	55	100	540,000	160,000
Staphylococcus albus	40,000	16,000	60,000	55	0	0	0
Pseudomonas fluorescens	30,000	1,000	12,000	55	10	60	9,000
Aerobacter aerogenes	12,000,000	14,000,000	10,000,000	60	2,800	35,000	75,000
Staphylococcus aureus	13,000,000	37,000	7,900,000	60	120	190	2,600
Bacillus subtilis	130,000	130,000	140,000	90	20	130	500
Salmonella pullorum	1,000,000	1,500	1,000	55	190,000	1,100	1,200

The data in table 7 show striking variations in the effect of water, 2-molal dextrose, and 2-molal sucrose on thermal resistance of cells. First, it will be noted that there is little or no evidence that either dextrose or sucrose afforded the strains of *Staphylococcus albus* or *Salmonella pullorum* any increased resistance to heat. However, it should be noted that the counts before heating in the dextrose and sucrose suspensions of *S. pullorum* are practically the same as those obtained after heating. When compared with the counts on the aqueous suspensions before heating it is evident that very large percentages of the organisms died almost instantly when introduced into the sugar solutions, but those which did survive were able to withstand the heat treatment.

There is only slight evidence that 2-molal sucrose protected *Bacillus subtilis*, whereas the results with *Pseudomonas fluorescens* and *Staphylococcus aureus* indicate protective action more definitely. *Serratia marcescens* and *Aerobacter aerogenes* were unmistakably protected by the presence of either dextrose or sucrose. Two-molal dextrose apparently offered more effective protection to *S. marcescens* than did an equimolal solution of sucrose; the reverse was true, however, for *A. aerogenes*. In each case in which protective action was observed the cells exhibited extreme sensitivity to heat in water suspensions.

EFFECT OF WASHING CELLS AFTER EXPOSURE TO SUGAR

Five cubic centimeters of an 18-hour plain broth culture of *Escherichia coli* 52 were centrifuged and all but 1 cc of the supernatant broth removed. One cubic centimeter of 100-percent sucrose was added and the resulting 50-percent sugar suspension of cells allowed to stand at room temperature for 30 minutes. The cells were then washed seven times with 0.85-percent NaCl solution to remove the sugar. At the appropriate time two 5-cc portions of a broth culture of the same organism were centrifuged to concentrate the cells. All but 1 cc of the supernatant broth was removed from one of these tubes and 1 cc of the 100-percent sucrose added as before. The cells were allowed to remain in contact with the sugar for 30 minutes at room temperature. The other tube of centrifuged cells was used as a broth control. In this case all but 2 cc of the supernatant broth was removed and the cells resuspended in the 2 cc volume of broth. After 1-cc portions of these three suspensions had been removed for plating before heating, the tubes were placed in the water bath at 54.5° C. for 5 minutes. Plates before and after heating were made with plain and dextrose agars.

TABLE 8.—*Thermal resistance of Escherichia coli 52 when exposed to 50-percent sucrose 30 minutes, then washed in 0.85-percent NaCl*

Treatment of cells	Agar	Count per cubic centimeter—		Survival per million
		Before heating	After heating at 54.5° C for 5 minutes	
Exposed to 50-percent sucrose 30 minutes, washed 7 times, and heated in 0.85-percent saline.	Plain	280,000,000	340,000	1,200
	Dextrose	320,000,000	410,000	1,300
Exposed to 50-percent sucrose 30 minutes, not washed, heated in the sucrose solution.	Plain	840,000,000	400,000,000	476,000
	Dextrose	900,000,000	400,000,000	444,000
Centrifuged from broth, resuspended, and heated in broth.	Plain	1,000,000,000	1,300,000	1,300
	Dextrose	960,000,000	4,200,000	4,400

It is quite evident from the data in table 8 that the protective action which 50-percent sugar afforded cells was readily removed by washing the cells in an 0.85-percent NaCl solution. The cells heated in saline and in broth suspensions were quite susceptible to heat, whereas the presence of sugar during heating resulted in increased resistance.

PROTECTIVE ACTION OF HYPERTONIC SOLUTIONS AGAINST THE COAGULATION OF NONLIVING PROTEIN SYSTEMS

It is obviously difficult to demonstrate changes in the physical status of the colloidal system of a minute bacterial cell except by indirect means. If the coagulation of cell colloids follows simple, well-defined laws of colloid chemistry, then the protective action afforded by sugars should be readily demonstrable with nonliving colloids. The following experiments were designed to investigate such a possible parallelism.

EFFECT OF HYPERTONIC DEXTROSE AND SUCROSE SOLUTIONS ON THE COAGULATION OF EGG ALBUMIN

By serial dilution of 8-molal sucrose and dextrose solutions, 6-, 4-, 3-, 2-, 1-, 0.5-, 0.2-, and 0.1-molal concentrations of each sugar were prepared. From these, series of tubes were arranged each containing 1 cc of one of the foregoing concentrations of sugar. To each tube 1 cc of fresh egg albumin was added and thoroughly mixed with the sugar solution, thus reducing the sugar to one half its original concentration. Tubes containing a mixture of equal parts of water and egg albumin, and also tubes contained undiluted egg albumin were used as controls.

The series of tubes for both sugars were placed simultaneously in a water bath at the desired temperature and the time of coagulation noted. It was necessary to adopt an arbitrary standard of turbidity for coagulation in order that the readings might be rendered uniform. The albumin was arbitrarily regarded as coagulated when it was no longer possible to identify and differentiate the letters on a typewritten page held behind the tube; the diameter of the tube was 10 mm.

TABLE 9.—*Effect of equimolal concentrations of dextrose and sucrose on the coagulation of egg albumin*

Molality of sugar	Time required to coagulate egg albumin at—					
	60° C.		65° C.		100° C.	
	Dextrose	Sucrose	Dextrose	Sucrose	Dextrose	Sucrose
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
4	(a)	(a)	(a)	(a)	0.5	0.5
3	(a)	(a)	34	52	.2	.2
2	73	(a)	8	11.5	.2	.2
1	23	31	4	4.5	.2	.2
0.5	8	10	3	3.5	.2	.2
0.2	6	7	3	3.5	.2	.2
0.1	6	7	3	3.5	.2	.2
0.05	5	5	2	2	.2	.2
Water, egg 1.1	3	3	2	2	.2	.2
Undiluted egg	4	4	2	2	2	.2

[a] Not coagulated after 24 hours.

The data in table 9 show the time required for coagulation of egg albumin at 60°, 65°, and 100° C. when mixed with equal parts of various equimolal concentrations of dextrose and of sucrose. At 60° a material increase in the time required for coagulation was observed when the sugars were present in 1-molal or greater concentrations. When 3-molal and 4-molal solutions of either sugar were employed coagulation was prevented for at least 24 hours. It is especially significant that sucrose afforded more protection than dextrose. The albumin coagulated in 73 minutes at 60° in the presence of 2-molal dextrose, whereas an equimolal solution of sucrose prevented coagulation for 24 hours.

When the experiment was repeated at 65° and 100° C. much more rapid rates of coagulation were observed. For example, with the 3-molal solutions the albumin failed to coagulate in 24 hours at 60°, but when exposed to 65° coagulation occurred in 34 minutes in the dextrose and 52 minutes in the sucrose solutions. At 100° coagulation in all concentrations employed occurred in relatively few seconds.

EFFECT OF VARIOUS SOLUTES ON THE INACTIVATION OF RENNIN BY HEAT

To a series of tubes each containing 2 cc of rennet extract was added an equal volume of one of the following substances: Conductivity water, 1-molal sodium chloride, 1-molal calcium chloride, 4-molal sucrose, and glycerol (specific gravity 1.25). The tubes, together with a control tube containing undiluted rennet, were placed in a water bath at 70° C. At various time intervals one drop of the rennet mixture from each tube was removed and added to tubes containing 10 cc of fresh, raw milk. The tubes of milk were held at room temperature and observed for the time of coagulation. Progressive inactivation of the rennin increased the time required to coagulate milk.

TABLE 10.—*Effect of various substances on the inactivation of rennin by heat*

[Material added to rennet, equal volume]

Period rennet mixture was exposed to 70° C. (minutes)	Coagulation time of 10 cc of milk by 1 drop of rennet mixture					
	Nothing added	Conductivity water	1-molal sodium chloride	1-molal calcium chloride	4-molal sucrose	Glycerol
	Minutes	*Minutes*	*Minutes*	*Minutes*	*Minutes*	*Minutes*
0	34	30	29	20	33	20
2.5	40	72	41	22	32	24
5.0	109	(a)	319	22	31	31
7.5	162		(a)	36	27	36
10.0	314			39	25	100
12.5	550			101	61	106
15.0	(a)			109	70	135
20.0				230	65	260
30.0				594	55	(a)
45.0				(a)	40	
60.0					35	
100.0					50	
120.0					50	
150.0					44	
180.0					70	
240.0					55	
300.0					60	

[a] Not coagulated after 24 hours.

It will be observed from the data in table 10 that 1 drop of the undiluted rennet before heating induced coagulation of 10 cc of milk in 34 minutes. After the milk had been heated for 2.5, 5.0, 7.5, 10.0, and 12.5 minutes, the coagulation times increased to 40, 109, 162, 314, and 550 minutes, respectively. After exposure to 70° C. for 15 minutes the rennin was so completely inactivated that it could not induce coagulation in 24 hours. All the milk not coagulated after 24 hours was still sweet to the taste.

The rennet extract which was diluted with water was completely inactivated after 5 minutes' exposure. This agrees with the decreased thermal resistance observed with certain bacterial cells in water suspension. Likewise, it will be observed in table 10 that 4-molal sucrose very effectively protected rennin against destruction by heat. Even after 5 hours' exposure at 70° C. the rennin was sufficiently active to coagulate milk in 60 minutes. Glycerol and 1-molal calcium chloride also afforded definite protective action, although not to the same degree as sucrose. Sodium chloride apparently hastened the inactivation of the rennin. The data for suitable control tubes were not incorporated in the table as the coagulation times were identical with those observed for the undiluted rennet, except in the case of the milk to which calcium chloride was added. As might be expected, the addition of calcium chloride to the milk reduced the time of coagulation a few minutes in each case. The observations are so completely in accord with those made with cell suspensions that the possibility of the operation of the same set of factors is forcibly impressed.

DISCUSSION

The data in this paper lend support to the concept that the individual cell does not die suddenly after a given exposure to heat. Unfavorable environmental factors induce changes in the cells, which behave in accordance with well-established principles of colloidal chemistry. It is believed that all the salient features of the data presented can be explained on the basis of the theory of cell destruction outlined by Bancroft and Richter (*2*). This concept permits of a logical basis for elucidating protective action, increased growth in carbohydrate media, sensitivity of cells to water, and other observations made in connection with this study.

The degree of dispersion of the colloids of the normal cell presumably is at least nearly optimum for maximum stability. As the agglomeration of the colloids becomes progressively more pronounced, the cell becomes more sluggish and eventually dormant. Such a cell is narcotized and may be revived if placed under conditions conducive to peptization of the colloids to their normal degree of dispersion.

It is apparent that the more advanced the degree of coagulation, the greater will be the difficulty encountered in peptization. Similarly, one should expect decided differences in the peptizing qualities of various media. The coagulated colloids of an injured cell may be peptized by one medium and not by another. Obviously, reversibility of coagulation is a relative matter and depends upon a complementary relationship with the peptizing qualities of the medium employed.

A summary view of the data presented in this paper suggests that certain sugars retard the agglomerating action of heat on the protoplasmic colloids. As a result, cells subjected to a given heat treatment in concentrated sugar solutions are only reversibly coagulated, whereas in aqueous suspensions the protoplasm more closely approaches the irreversible stage of coagulation. In these experiments thermal exposures have been employed which were barely adequate to kill the cells in aqueous suspensions, thereby favoring a demonstration of the maximum protective action in the sugar suspensions. Experiments are now in progress with cultures whose heat resistance is just at the threshold of the thermal exposure of pasteurization. If some of the organisms in ice-cream mix are sufficiently protected by the presence of sugar to enable them to survive, the need for a readjustment of pasteurization requirements for this product would be suggested. The practical aspect of the protective action of sugar is also manifested in the production of sweetened condensed milk, and the preservation of foods containing high percentages of sugar such as fruits canned in sirup, honey, molasses, etc.

The protective action of hypertonic sugar solutions against the action of heat on egg albumin and rennin further suggests that the protection is afforded by retarding the rapid coagulation of the colloidal complex. The addition of water hastened the time of coagulation or destruction of the enzyme, and the addition of hypertonic sugar solutions retarded the same process. The parallelism between the observation with nonliving proteins and those made with living cells is quite apparent. The precise mechanism by which sugar solutions retard the coagulation of nonliving proteins is one which still challenges the attention of physical chemists. The data presented in this paper suggest that the same fundamental principles involved may apply to the destruction of bacteria.

When the cell colloids have agglomerated to the extent that all efforts fail to peptize them, the cell may be regarded, with reservations, as irreversibly coagulated or dead. The impracticability of proving such a point beyond all peradventure of doubt is illustrated by the many reports in the literature of delayed germination of heated cells. Although it is quite proper to conclude that the colloids of a cell are irreversibly coagulated with respect to a given medium, obviously the conclusion should be confined to the observation, especially if minimal exposures have been employed. Any determination of minimum lethal exposure is subject to question on the basis of the uncertainty of the death of the organism.

The importance of permeability in protective action is suggested by the results of several experiments. Prolonged exposure of certain cells to hypertonic solutions results in an actual increase in the number of cells capable of withstanding a given heat treatment, although the total number of cells in the unheated samples may show a steady decline. It is believed that this is explicable on a basis of different degrees and rates of permeability of the cells in the population. The variations observed in the protective action of various sugars for different organisms also suggest that penetration of the cell wall must play an important role in the regulation of protective action.

When certain cells are heated in a series of solutions of dextrose with increasing osmotic pressures, there is an unmistakable parallel increase in the protective action. A similar series of equimolal concentrations of sucrose will show considerably greater protective action than the dextrose solutions. This suggests that although osmotic pressure is an important factor in the protective action afforded by sugars it is not the only agency involved. If the protective action is to be explained on a basis of the transfer of water, obviously osmotic pressure would play an important part. On the other hand, the effective osmotic pressure at the cell surface is regulated not only by the molecules in solution but by the relative permeability at each individual cell surface.

SUMMARY AND CONCLUSIONS

This work involves a study of the increased resistance manifested by certain micro-organisms when heated in the presence of high concentrations of sugars. The significance of this phenomenon in the heating of ice-cream mix is demonstrated and the probable relation to the preservation of condensed milk, canned fruits, honey, molasses, etc., is suggested.

Within the limitations imposed by the experimental procedure it has been possible to demonstrate some of the fundamental points bearing on the mechanism of this protective action. The permeability of the individual cell apparently plays an important part in regulating the rate of death in water and in sugar suspensions.

The protective action increased with increased osmotic pressure in a series of concentrations of a given sugar, but equimolal solutions of different sugars did not show the same protective action. Maltose and lactose gave little or no protection to the cells studied.

Not all cells exhibit the phenomenon of increased thermal resistacne in hypertonic solutions. Such resistance is believed to be limited to those cells which are highly sensitive to water at slightly increased temperatures. Washing of cells after exposure to hypertonic solutions removes any protective action which the sugar solution affords the cells in the unwashed portion.

Parallel with the protective action for cells, hypertonic sugar solutions have been shown to delay or even prevent the coagulation of egg albumin and to retard the inactivation of rennin by heat.

The general conclusions from these data may be briefly stated as follows: The addition of sugar to ice-cream mix may increase the thermal resistance of the microflora. Hypertonic solutions of dextrose and sucrose made up in broth, milk, water, or ice-cream mix afforded certain cells definitely greater protection against heat than when heated in water suspensions. The addition of sugar after heating did not induce an increased survival of the cells employed in this study. The variation in the peptizing qualities of different media for the cells which have been only reversibly coagulated by minimal exposures suggests caution in all studies based upon the death of the cell as measured by cultural methods.

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THE INFLUENCE OF LIME ON THE REACTION OF SUBSOILS[1]

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INTRODUCTION

Much has been learned of the effect of lime on the soil, especially on the part that is constantly being stirred by the plow and other implements of cultivation. Less is known, however, of its effect on the soil that lies immediately beneath the plowed depth. Work at Rothamsted, England,[2] at Cornell,[3] Florida,[4] and other experiment stations has shown that lime is lost rather rapidly from the soil through drainage waters. It is reasonable to suppose that as the lime-charged water percolates through the subsoil, it gradually neutralizes any acids that may be present and thus raises the pH value.

The soil-fertility plots at the New Jersey Agricultural Experiment Station seemed to offer an excellent opportunity for obtaining some definite information on this point. The soil involved is the Sassafras loam and is of fair quality only. It can scarcely be called typical Sassafras soil, since it occurs just at the southern boundary of the soil which is derived from the Triassic red shale. Little is known of the history of this soil previous to 1908, when the plots were laid out and the experimental work was begun. It is quite certain, however, that lime had not been applied for many years and possibly not at all.

During the period of the experimental work certain of these plots have received no commercial lime. Others have been limed regularly at 5-year intervals (carbonate form), some receiving 2 tons to the acre, some 1 ton, and others ½ ton. In all the experiments the land was plowed to a depth as nearly uniform as possible under the circumstances, about 7 inches.

NITROGEN-AVAILABILITY PLOTS

The nitrogen-availability series of plots contains forty $\frac{1}{20}$-acre plots. Half of these have received no lime during the experimental period and the other half have been limed at the rate of 2 tons of the carbonate form to the acre, at 5-year intervals. The last application before the samples under consideration were taken was made in the spring of 1928. Samples of soil were collected from these plots in September 1932. The top or surface sample was taken to a depth of $6\frac{2}{3}$ inches; the second $6\frac{2}{3}$ inches represents the subsoil. The samples were prepared and pH determinations made by the quinhydrone method.

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The results, together with the fertilizer treatments for the plots, are presented in table 1. A study of the table shows that with few exceptions the pH values of the samples from the unlimed plots range from about 5.0 to 5.8, while the pH values of the samples from the limed plots range from a little less than 7 to about 7.5. The subsoils from the unlimed plots are just about as acid as the topsoils. On the other hand, the acidity of the subsoils from the limed plots has been corrected to just about the same extent as the acidity of the surface soils. The figures given in table 1 seem to furnish conclusive proof that the lime, in its journey downward, does neutralize the acidity of the subsoil and thus raise the pH value.

TABLE 1.—*pH values of surface soils and subsoils from nitrogen-availability plots, 1932*

Plot no.	Fertilizer treatment (1/20 acre)	Unlimed section		Limed section	
		Surface soil	Subsoil	Surface soil	Subsoil
		pH	*pH*	*pH*	*pH*
1	None	5.12	5.56	7.55	7.50
2	16 pounds muriate of potash	5.03	5.67	7.45	7.13
3	32 pounds superphosphate	5.09	5.52	7.18	7.15
4	Minerals only [1]	5.23	5.51	7.11	7.22
5	Minerals and 1,600 pounds cow manure	5.78	5.62	7.06	7.10
6	Minerals and 1,600 pounds horse manure (manure discontinued after 1922)	5.32	5.47	6.78	6.68
7	None	4.70	5.30	6.87	7.10
8	Minerals and 8 pounds $NaNO_3$	5.54	5.62	6.88	7.09
9	Minerals and 16 pounds $NaNO_3$	5.50	5.80	7.16	7.20
10	Minerals and $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$	5.49	5.70	7.14	7.30
11	Minerals and $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$	4.12	4.67	6.00	6.37
12	Minerals and $CaCN_2$ equivalent to 16 pounds $NaNO_3$	5.70	5.58	7.24	7.37
13	Minerals and dried blood (discontinued after 1922)	5.23	5.53	7.05	6.90
14	Minerals, $NaNO_3$, and $(NH_4)_2SO_4$ (half the nitrogen from each)	5.30	5.66	6.90	6.95
15	Minerals and concentrated tankage	5.02	5.27	6.80	6.95
16	Minerals only	5.08	5.06	6.80	7.00
17	do	5.40	5.46	7.09	6.85
18	Minerals, 1,600 pounds cow manure, and 16 pounds $NaNO_3$	6.06	5.82	7.35	7.32
19	Minerals only	5.15	5.23	7.10	7.28
20	Minerals, 200 pounds wheat straw, and 16 pounds $NaNO_3$	5.80	5.65	7.47	7.45

[1] Minerals = 16 pounds superphosphate and 8 pounds muriate of potash.

CALCIUM AND MAGNESIAN LIMESTONE PLOTS

In a second series of plots representing the same type of soil as the first, magnesian limestone, and high-calcium limestone have been applied over a period of 25 years, at 5-year intervals, in amounts of ½ ton, 1 ton, and 2 tons to the acre. The crops have been largely grain, hay, and forage, though vegetables were grown for a number of years on one section.

Samples of soil were collected from these plots for pH determinations in July 1933. Table 2 shows the pH readings for the surface and subsoils from four different sections that received the lime treatments. An examination of the table shows at once a corresponding rise in the pH value of the surface soil and subsoil as the lime was increased.

Table 2.—*pH values of surface soils and subsoils when different lime treatments were applied and different crop rotations grown*

Lime treatment	Rotation 1, plots 21-27, general farm crops		Rotation 2, plots 28-34, general farm crops		Rotation 3, plots 35-41, vegetable crops		Rotation 4, plots 42-48, forage crops and hay	
	Surface soil	Subsoil	Surface soil	Subsoil	Surface soil	Subsoil	Surface soil	Subsoil
	pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
No lime	5.16	5.20	5.48	5.70	5.02	5.17	4.92	5.14
1,000 pounds calcium limestone	5.73	5.70	5.68	6.05	5.10	5.46	5.06	5.58
2,000 pounds calcium limestone	6.07	6.06	6.20	6.36	5.69	6.08	5.75	6.02
4,000 pounds calcium limestone	7.17	7.00	7.20	7.17	6.40	6.92	6.87	7.00
1,000 pounds magnesian limestone	5.78	6.00	6.00	6.05	5.62	5.56	5.73	5.70
2,000 pounds magnesian limestone	6.43	6.54	6.30	6.50	6.20	6.34	6.15	6.16
4,000 pounds magnesian limestone	7.44	7.16	7.46	7.20	7.00	7.22	6.97	7.10

The pH values for the different rotations are generally in fair agreement. For rotations 2, 3, and 4, there is a slight tendency for the pH of the subsoils to be higher than that of the surface soils. Almost without exception the plots that received no lime, both soil and subsoil, have low pH values.

With only one exception the pH values for the magnesian limestone are somewhat higher than those for the corresponding calcium limestone treatments. This is to be expected, since the limestones were used in equal weights rather than in equivalent amounts of actual lime (CaO).

CONTINUOUS WHEAT AND RYE PLOTS

In September 1932 samples of soil were collected for pH determinations from plots on which wheat and rye had been grown continuously since 1909. Lime had been applied usually at 5-year intervals to maintain the reaction at about pH 6.5 to 7. The pH determinations are shown in table 3. The eight samples of surface soil show a range of pH 6.10 to 6.42, and the eight subsoils a range of 5.96 to 6.39. In each case the pH value of the subsoil was only slightly lower than that of the surface soil. A sample of surface soil and subsoil from an adjoining unlimed plot had pH values of 5.5 and 5.38, respectively. In this case the pH value of the subsoil from the limed plot was about 0.8 higher than that of the subsoil from the unlimed plot.

Table 3.—*pH values of surface soil and subsoil from plots continuously in wheat and rye, 1909-32*

Plot no.	Crop	pH value of surface soil	pH value of subsoil
68	Rye	6.42	6.39
68-A [1]	do	6.42	6.28
69	Wheat	6.31	6.30
69-A [1]	do	6.31	6.30
70	Rye	6.33	6.20
70-A [1]	do	6.23	6.00
71	Wheat	6.25	6.13
71-A [1]	do	6.10	5.96

[1] The A plots receive each year a top-dressing of nitrate of soda at the rate of 160 pounds to the acre.

LEGUME AND NONLEGUME PLOTS

In series 1 there were eight $\frac{1}{20}$-acre plots which had been in a 5-year rotation for 15 years. Lime had been applied at 5-year intervals to maintain the reaction near the neutral point. The crops grown were grain and hay. One half the plots were kept in legumes, when the rotation would permit, and the other half in nonlegumes. Samples of soil were collected from these plots for pH determinations in October 1932. The results are shown in table 4.

TABLE 4.—*pH values of surface soil and subsoil from limed plots in legume and in nonlegume rotations*

SERIES 1

Plot no	Rotation	pH value of surface soil	pH value of subsoil
73	Nonlegume	7.35	7.20
74	Legume	7.50	7.15
75	Nonlegume	7.38	7.21
76	Legume	7.38	7.12
77	Nonlegume	7.45	7.20
78	Legume	7.55	7.32
79	Nonlegume	7.52	7.30
80	Legume	7.30	7.20

SERIES 2

Plot no	Rotation	pH value of surface soil	pH value of subsoil
1	Nonlegume	7.05	7.10
2	do	6.90	6.84
3	Legume	6.48	6.50
4	do	6.72	7.00

With slight exception the eight surface soils show pH values about 0.15 to 0.25 higher than the subsoils. There appears to be no correlation between the soil reaction and the rotation practiced. Samples from unlimed plots adjoining or near the experimental plots gave pH values of about 5.2 to 5.3. Here is a difference of about 2 points between the unlimed surface and subsoils and the corresponding limed soils. It is evident that the lime has influenced the reaction of the soil to a depth of at least 12 to 13 inches.

In series 2 there were 4 plots, 2 of which had been in a nonlegume rotation for about 15 years and 2 in legumes, or legume cover crops, when the rotation would permit. Lime had been applied to keep the soil near the neutral point. Samples of soil were collected for pH determinations in October 1933. The pH values are shown in table 4. On nearby plots without lime the pH value of both surface and subsoil was about 5. It is evident that here, too, the lime had worked downward and changed the reaction of the subsoil to about the same degree as the surface soil.

ALFALFA PLOTS

Four plots had been in alfalfa 12 years during the past 20 years (1914 to 1918, inclusive; 1922; 1925 to 1927, inclusive; 1931 to 1933, inclusive). For the remaining years the crops were corn, oats, wheat, and potatoes. One of these plots had not been limed during the entire 20 years; another had been limed (carbonate form) at 5-year intervals at the rate of 1,000 pounds per acre; a third had been limed at the rate of 2,000 pounds per acre, and a fourth at the rate of 4,000 pounds per acre. Samples of surface soil and subsoil were collected for pH determinations in July 1933 (table 5).

TABLE 5.—*pH values of the surface soil and subsoil of limed and unlimed plots that had been in alfalfa 12 years during the past 20, and yields of alfalfa hay on the same plots, 1931–33*

Plot no	Lime treatment (pounds limestone per acre)	Yield of hay per acre				Surface soil, 1933	Subsoil, 1933
		1931	1932	1933	Average		
		Pounds	*Pounds*	*Pounds*	*Pounds*	*pH*	*pH*
1	None	1,713	650	1,861	1,408	5.13	5.24
2	1,000	4,458	2,082	3,913	3,494	5.32	5.31
3	2,000	7,868	3,988	6,925	6,260	5.55	5.55
4	4,000	9,211	5,097	8,087	7,465	6.68	7.05

On the unlimed plot both the surface soil and the subsoil were strongly acid, just above pH 5. On this plot weeds and grasses have practically driven out the alfalfa. On the limed plots the pH values of the subsoil, with the exception of plot 4, were approximately the same as those of the surface soil, and the pH values rose as the lime was increased.

The yields of alfalfa hay for the years 1931 to 1933, inclusive, are shown (table 5) for the purpose of emphasizing the relation between the reaction of the soil and the yield of hay.

SUMMARY

An attempt has been made to determine the extent to which lime, in the carbonate form, applied to the surface soil, works downward and changes the reaction of the subsoil.

The soil used in the tests was a Sassafras loam taken from the soil-fertility plots of the New Jersey Agricultural Experiment Station. Certain of these plots have been limed at 5-year intervals for 25 years; others have received no lime. Samples representing the top 6⅔ inches and corresponding subsoil samples representing the stratum from 6⅔ inches to about 13 inches were collected and the pH values determined.

With few exceptions the figures show that the subsoils from the unlimed plots gave pH readings only a little higher than the corresponding surface soils. In most cases there was very little difference between the pH values of the surface and subsoil from the limed plots. Both the surface and subsoil of these plots gave pH readings about 1½ to 2 points higher than those of the corresponding unlimed plots.

It is thus established that in the case of this particular soil carbonate of lime used at the rate of 2,000 to 4,000 pounds to the acre over a period of 25 years has changed the reaction of the subsoil (6⅔ to 13 inches) to about the same degree that it has changed the reaction of the surface soil. It seems reasonable to conclude, therefore, that in general where generous applications of lime are made on acid soils, the acidity of the subsoil will also be gradually corrected.

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GROWTH AND INJURIOUS EFFECTS OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA[1]

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INTRODUCTION

A knowledge of the rate of canker growth and development constitutes a basic element in the study of *Cronartium ribicola* Fischer on its aecial hosts and is of fundamental importance in determining the age of infection (*4*)[3] on *Pinus monticola* Doug. and in considering the rate of damage. Studies of the growth and injurious effects of cankers have therefore been an essential part of the general investigations of the rust on *P. monticola* as carried out by the Portland branch of the Division of Forest Pathology since the discovery of the disease in the native range of this species in the western part of North America in 1921 (*3*).

In these investigations canker is defined as including all stages in the development of the individual infection, from the appearance of the first recognizable discoloration that marks the first symptoms of the disease in the bark. While hyphae are generally present in the bark before the appearance of discoloration, the limits of the discoloration mark rather definitely the advance of the mycelium (*2, p. 625*). Measurements of the extent of the visible symptoms are therefore sufficient for practical purposes.

METHODS

Cankers mainly in the younger stages were selected for growth measurements so that subsequent development might be followed for a number of years. The extremes of length and width of the discolored area were marked at the beginning of the growing season with white lead,[4] a marking material that was found by Rhoads to be satisfactory in studies of blister rust canker growth on *Pinus strobus* L in 1918 (*6*). After marking, measurements of the extensions of the discoloration beyond these lines were recorded periodically at the end of the growing season in the fall and at the beginning of general growth activity the following spring. Only cankers with clearly defined limits were considered.

It was recognized that such factors as the formation of new cankers, coalescence of cankers, and gnawing of the infected bark by squirrels would render some of the cankers useless for later measurements. Therefore additional cankers were marked to provide against this loss, and all cankers were labeled at first with only temporary labels.

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[1] Received for publication Sept. 15, 1933; issued June, 1934.

[2] In the collection of the data on which this paper is based the writer was assisted by C N. Partington, junior pathologist, and J. L. Mielke, assistant pathologist, Division of Forest Pathology.

[3] Reference is made by number (italic) to Literature Cited, p 502

[4] White Duco was used in some of the later experiments reported here and was found to be a better marking material than white lead.

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Those suitable for study were permanently labeled when the first measurements were taken.

Data taken for each of the cankers measured were as follows: Place; date; tree number; height of tree at time of marking and subsequent growth in height; condition of cankers and number on tree; serial number of canker and location on tree (i.e., whether on branch or trunk); diameter below canker, growth rate, and condition of infected branch or trunk; age of internode on which canker started; length and width of cankered area at time of marking; age of canker, as nearly as could be determined; subsequent canker growth upward, downward, to left, and to right; and general notes on growth of cankers, production of fruiting stages, etc. The initial measurements were taken for the greatest lengths and widths of the cankers, and the same principle was followed for all subsequent measurements. In general, the latter fell in line with the first measurements, but frequently an irregular extension threw the lines of measurement off the original axes.

Trees selected in the study were mainly thrifty young specimens ranging between 5 and 25 feet in height. Cankers were marked on twigs, branches, and trunks of all diameters from the smallest to the largest and of all conditions, from thrifty to suppressed.

Growth rates established by these measurements were supplemented by the observed development of the marked cankers and by observational analyses of the extent of growth and of the effect of large numbers of other cankers of all sizes and ages on all classes and conditions of trees throughout a much wider range, thus providing the basis for the conclusions on injurious effects of canker growth.

BASIS OF COMPARATIVE STUDIES OF CANKERS IN VARIOUS AREAS

Although *Cronartium ribicola* is now widely distributed throughout most of the range of *Pinus monticola* in the Pacific Northwest [5] (*4, p. 1*), at the time the growth studies were begun the main range of the disease was centered in southwestern British Columbia. Therefore most of the areas in which measurements were made are located in this section. The areas were selected to represent site and climatic conditions generally favorable to the growth of *P. monticola* and at the same time to provide a range in these conditions that would give a measure of the effect of site and climate upon canker growth. Accordingly some of the areas are situated near sea level, where the growing season is relatively long, and others are located at higher elevations and more inland points, where the season is materially shorter. All are in British Columbia.

The following areas were selected: Chee Kye, near sea level on the Cheakamus River about 50 miles north of Vancouver; Bold Point, on Quadra Island, and Thurston Bay, on Sonora Island, both at sea level about 110 and 130 miles, respectively, northwest of Vancouver; Daisy Lake, at an elevation of 1,300 feet in the Coast Range about 60 miles north of Vancouver; Mile 72, on the Pacific Great Eastern Railway, at an elevation of 1,300 feet in the Coast Range about 110 miles north of Vancouver; Revelstoke, at an elevation of 1,500 feet on the Columbia River about 250 miles east of Vancouver; and a locality near Arrow Park, at an elevation of 1,700 feet on the Arrow Lakes about 60 miles south of Revelstoke. At the first three places growth rates of *Pinus monticola* are closely comparable to those in the optimum range of the species in northern Idaho and western Montana. At the last four places the growing season is normally 1 month to 1½ months shorter than at the first three places, and growth is generally slower.

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[5] Pacific Northwest as used in this paper includes southern British Columbia, Washington, Oregon, Idaho, and western Montana.

At the beginning of the growing season in the spring of 1923 about 100 cankers were marked at each of the above-named places except Revelstoke and Arrow Park. The great size of the unopened territory covered in the investigations during this year made it impossible to secure measurements in the fall except at Chee Kye and Thurston Bay. Measurements were made in the spring of 1924, approximately 1 year after the marking, at all places except Mile 72, where the trees had been destroyed in a forest fire which swept that section in the fall of 1923. Trees in the Bold Point area were likewise destroyed by fire in 1924. In the other areas measurements were not attempted after the spring of 1924, because of the death of the cankers or complications of their growth from various causes.

At Revelstoke 74 cankers were marked in the spring of 1928 and measured in the fall. Those that were not complicated by coalescence of cankers, squirrel feeding, etc., were remeasured in the fall of 1929. At Arrow Park 138 cankers were marked in October 1929 and measured at the start of the growing season in the spring of 1930 and again at the end of the growing season in the fall.

A special study of the growth of trunk cankers was made at Chee Kye and Daisy Lake on cankers marked in the fall of 1925. Measurements of these were made at Daisy Lake in the spring of 1926 and the fall of 1926 and of 1927, and at Chee Kye in the fall of 1926 and of 1927.

Because of the complicating factors enumerated, only a part of the cankers that were marked remained suitable for measurements in most of the areas. As already stated, the three localities—Chee Kye, Bold Point, and Thurston Bay—are representative in growth characteristics of the optimum for *Pinus monticola* and are similar in general growth conditions for the rust. The size and condition of the trees and branches on which the cankers were marked were similar in all areas. The trees were thrifty, young, open-grown specimens, and the stems bearing the marked cankers were generally in thrifty or good condition. As was to be expected, therefore, the individual growth performances and general growth averages of the cankers at these places were generally comparable. In view of the general similarity of these areas, the values for these three are capable of combination.

The measurements made at the same time at Daisy Lake showed a much slower growth of cankers than in the three other areas. Canker growth was likewise slower at Revelstoke and Arrow Park. But while the general site conditions for canker growth of these three areas are fairly comparable, differences in the character of the cankers and of the trees and branches on which they occurred make the values from them unsuitable for combination, and therefore each must be considered separately.

At Daisy Lake the marked cankers were located primarily on the smaller branches that were in only fair or in poor condition, in the lower crowns of rather large trees, or on suppressed smaller trees. At Revelstoke only branch cankers were marked. In general, the cankers were older at the time of marking than were those at the other places. The affected branches at Revelstoke soon entered a stage of such rapid decline that most of them died from the canker outward to the tip during the following season. The cankers marked at Arrow Park were all branch cankers. In general, these cankers and the trees and branches on which they occurred were comparable in size and relative thrift with those in the optimum areas. They offer a good opportunity, therefore, for comparison with those of the optimum areas for canker growth under contrasting climatic and site conditions. Further comparison of growth under such conditions is available primarily from the special study of trunk cankers at Daisy Lake and Chee Kye from 1925 to 1927.

GROWTH OF CANKERS

RATE OF GROWTH IN RELATION TO SIZE AND VIGOR OF STEM

At the beginning of the investigations it was evident from general observations of cankers of similar age that the rate of canker growth varied decidedly with the size and, to some degree, with the vigor of the affected part. Rhoads, on the basis of growth measurements of the rust on *Pinus strobus* in 1918, stated (*6, p. 517*): "In general it may be said that the growth of the blister rust cankers is in direct proportion to the size and vigor of the stems on which they occur." He offered no concrete evidence, however, on the influence of vigor, and in his table 1 he has combined branch, twig, and trunk cankers without reference to vigor. What part vigor plays in the values he has shown for correlation with size is therefore uncertain. From the present study it appears that the factor of size, corresponding with the amount of bark tissue available, entirely predominates over that of vigor; therefore, Rhoads' values are probably fairly indicative of the size factor.

In studying the influence of vigor on cankers it was thought best in the present work to consider twig and branch cankers separately from trunk cankers, since the growth of the trunks and leaders is normally more rapid and vigorous than that of the branches. In the classification of trunks and leaders, as compared with branches and twigs in *Pinus monticola*, a further distinction is naturally suggested by the characteristic differences in their general habit of growth. The trunks are normally straight and erect, whereas the branches are horizontal and more or less curved; the internodes of the trunks are longer and taper more slowly than those of the branches and the nodes of the trunks are distinctly marked by the regular radially whorled arrangement of the branches, whereas on the branches the habit of branching is flatter and more irregular and the nodes are less distinctly marked. Normally, a trunk of given diameter will be considerably younger and more active in growth than a branch of equal size.

Comparisons were made of canker growth in the same place and at the same season on trunks and branches of equal size. Provided the condition of the branches and trunks was fair or better, no essential differences between them in growth rate of cankers were evident; therefore, the more rapid growth and greater vigor of the trunks was without apparent influence. In this connection it should be stated that the bark, which is available for mycelial growth, is slightly thicker on the branches than on the trunks, which are younger though of equal size. Conditions for canker growth, therefore, were a little more favorable on the branches than on the trunks. On the other hand, if this slight difference in bark thickness offsets the difference in vigor, it would appear that the influence of the latter was, at the most, comparatively slight. It was evident, therefore, that vigor or lack of vigor would have to be extreme in order to have a material influence upon the growth of the fungus.

On the other hand, the bulk of the living bark tissues, as indicated by the size of the part infected, was clearly the most essential condition of the host for rapid growth of the canker.

ANNUAL LONGITUDINAL GROWTH

INFLUENCE OF SIZE OF STEMS

The influence of the size of the part infected on canker growth is shown in table 1, which gives, by diameter classes of the part infected, the average annual longitudinal growth of branch and trunk cankers at Chee Kye and Thurston Bay and of branch cankers at Bold Point, measured in the spring of 1924. Growth rates at Chee Kye and Thurston Bay were so nearly identical that the data for these areas are combined. Canker growth at Bold Point was somewhat more rapid, and since the measurements here consisted almost entirely of branch cankers, the values for this area are given separately.

TABLE 1.—*Average longitudinal growth, April 1923 to April 1924, of branch and trunk cankers at Chee Kye and Thurston Bay and branch cankers at Bold Point, British Columbia*

Stem-diameter class (inches)	Chee Kye and Thurston Bay						Bold Point		
	Branch cankers			Trunk cankers			Branch cankers [a]		
	Cankers	Average branch diameter	Canker growth	Cankers	Average trunk diameter	Canker growth	Cankers	Average branch diameter	Canker growth
	Number	*Inches*	*Inches*	*Number*	*Inches*	*Inches*	*Number*	*Inches*	*Inches*
0.1									
0.2-0.3	20	0.28	4.12				10	0.28	3.93
0.4-0.5	21	.43	4.50	1	0.50	4.90	15	.47	5.13
0.6-1.0	6	.62	5.63	3	.83	5.60	27	.73	6.39
1.1-1.5				4	1.33	6.20	5	1.32	7.20
1.6-2.0				8	1.76	7.50			
2.1-2.5									
2.6-3.0				2	2.90	8.45			
3.1-3.5									

[a] In addition, measurements were secured on 1 trunk canker, stem diameter 2.5 inches, canker growth 8.6 inches.

The increase in the rate of canker growth with size of the part infected is striking. The curve of growth rate over size of part infected is parabolic in form. This is indicated in figure 1, in which the Chee Kye and Thurston Bay data of table 1 are plotted. The short curve for the growth of branch cankers is so close to the beginning of the much more extended curve for the growth of trunk cankers that for all practical purposes it may be regarded as an extension of the longer curve. Under the conditions found at Chee Kye and Thurston Bay, from April 1923 to April 1924, which may be considered fairly representative of the optimum, the growth rate of cankers increases very rapidly for the smaller stem-diameter classes, attaining an average of 6 to 8 inches a year for stem diameters from 1 to 2 inches.

For growth rates at still larger diameters under these conditions, it is necessary to consider the measurements of 1926 of the trunk cankers from the Chee Kye area. The year from October 1925 to October 1926 covered by these measurements, was comparable in seasonal conditions for canker growth to the year from April 1923 to April 1924, covered by the branch- and trunk-canker measurements at Chee Kye and Thurston Bay. The condition of the cankers and the class and condition of the trees on which they occurred were likewise comparable for the two sets of measurements. The 1926 data at Chee Kye shown in table 2 therefore constitute a natural extension of the 1924 data given in table 1.

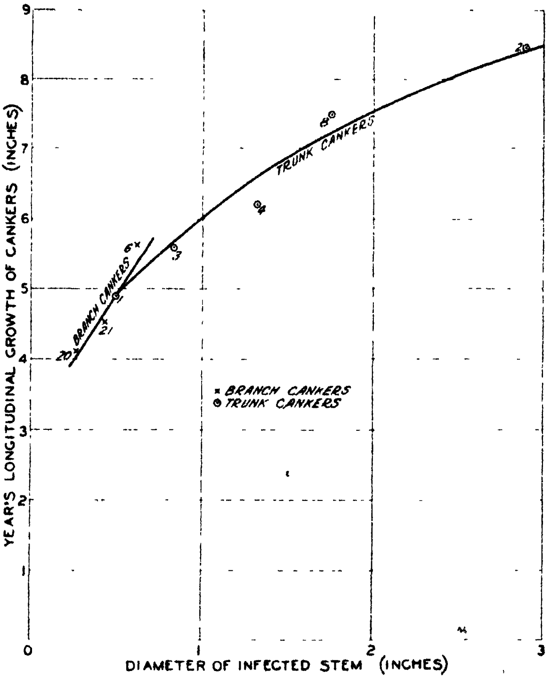

FIGURE 1. Average longitudinal growth from April 1923 to April 1924 of cankers marked at Chee Kye and Thurston Bay, British Columbia, in April 1923

From table 2 it is evident that the increase in growth rate of cankers tapers off very rapidly in the larger stem-diameter classes. This tendency and that of the 1926 Chee Kye data to coincide with and extend the 1924 Chee Kye and Thurston Bay values are still more evident from figure 2, in which the curve of the 1926 Chee Kye values is given together with the curves of the 1924 Chee Kye and Thurston Bay data.

TABLE 2.—*Average longitudinal growth from Oct. 31, 1925, to Oct. 16, 1926, of trunk cankers marked near Chee Kye, British Columbia, Oct. 31, 1925*

Trunk-diameter class (inches)	Cankers	Average trunk diameter	Canker growth	Trunk-diameter class (inches)	Cankers	Average trunk diameter	Canker growth
	Number	*Inches*	*Inches*		*Number*	*Inches*	*Inches*
2.6–3.0	2	2.85	8.15	6.1–7.0	3	6.47	8.83
3.1–4.0	10	3.56	8.98	7.1–8.0	1	7.90	9.10
4.1–5.0	6	4.60	10.15	11.1–12.0	1	11.80	9.50
5.1–6.0	3	5.33	8.37				

Beyond the 2-inch stem-diameter class the longitudinal growth rate of cankers increases very much more slowly, and beyond the 4-inch or 5-inch stem-diameter class it tends to become stabilized or constant. In the data under consideration, representing optimum conditions, the maximum or constant rate of canker growth averages between 9 and 10 inches a year.

INFLUENCE OF VIGOR OF STEMS

The relative condition of the branches and trunks on which the cankers forming the basis for tables 1 and 2 and figures 1 and 2 occurred was characterized as poor, fair, good, and thrifty. In the main, the condition was either good or thrifty. Since no differences in growth rate attributable to differences in thrift were evident between branch cankers and trunk cankers, an effort was made to establish as far as possible the extent of the influence of this factor by comparison of these cankers according to the vigor classification of the trunks or branches. These classifications were based on the growth rate and apparent condition of the branch or trunk during the year from time of marking to time of measurement. Each canker occurring on a stem that at the time of measurement was classified as poor or fair was paired with the nearest listed canker of most nearly comparable stem diameter, in the same area and year of measurement, occurring on a stem classified as thrifty. No canker was used twice. In case a canker in the thrifty-stem classification was not available for comparison, the nearest canker occurring on a stem classified as good was used. The longitudinal growth of these mathematically paired cankers is compared in table 3. The values do not indicate that differences in the vigor of the stems had any influence on the growth rates of the cankers. The averages are practically identical. The only decided influence is that of size of the part infected.

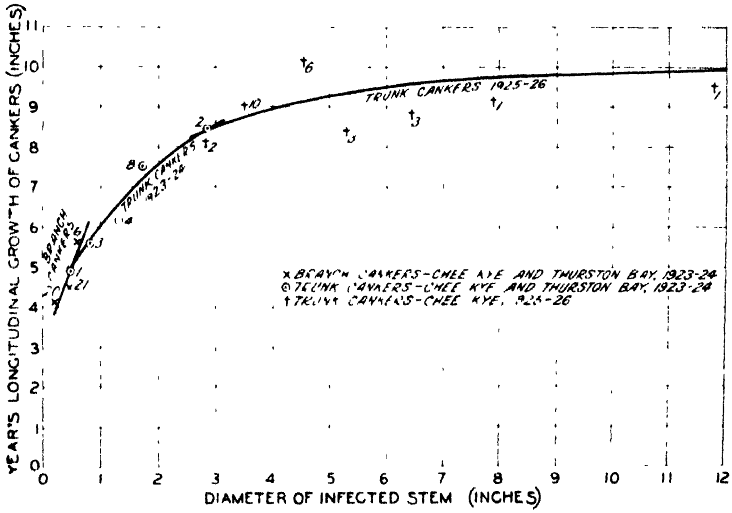

FIGURE 2. Longitudinal growth rates of *Cronartium ribicola* on *Pinus monticola* under optimum conditions at Chee Kye and Thurston Bay, British Columbia.

TABLE 3.—*Comparison of longitudinal growth of cankers on stems in poor or fair condition with that of cankers on stems of similar size but in thrifty or good condition at Chee Kye and Thurston Bay, British Columbia*

Pair of cankers compared (no.)	Stem in poor or fair condition: Stem diameter	Stem in poor or fair condition: Condition of stem[a] At marking	Stem in poor or fair condition: Condition of stem[a] At measuring	Stem in poor or fair condition: Canker growth	Stem in thrifty or good condition: Stem diameter	Stem in thrifty or good condition: Condition of stem[a] At marking	Stem in thrifty or good condition: Condition of stem[a] At measuring	Stem in thrifty or good condition: Canker growth
	Inches			*Inches*	*Inches*			*Inches*
1	0.2	F	P	4.0	0.2	T	G	4.4
2	.2	F	F	4.8	.3	G	G	4.8
3	.3	P	P	4.3	.3	T	T	4.3
4	.3	F	P	4.1	.3	G	G	3.6
5	.3	F	F	3.4	.3	G	G	4.4
6	.4	F	F	4.5	.4	G	G	4.7
7	.4	G	P	3.7	.4	G	G	3.9
8	.5	F	F	6.1	.4	T	G	4.7
9	3.0	T	P	7.6	2.7	T	T	8.7
10	3.2	G	F	9.9	3.2	T	T	8.5
11	3.4	G	F	8.0	3.4	G	T	10.0
12	3.5	T	F	8.6	3.6	T	T	8.9
13	4.0	G	F	9.3	4.0	T	T	9.1
14	4.6	G	F	9.8	4.6	T	T	9.0
15	5.0	G	F	10.6	5.2	T	T	7.7
16	5.5	P	P	8.8	5.3	T	T	8.6
17	6.4	T	F	9.1	6.8	T	T	8.3
Total	41.2	3 T, 6 G, 6 F, 2 P	11 F, 6 P	116.6	41.4	11 T, 6 G	10 T, 7 G	113.6
Average	2.42	G-F	F-P	6.86	2.44	T-G	T-G	6.68

[a] P=poor; F=fair, G=good, T=thrifty

Evidently, under ordinary conditions these vigor classifications do not sufficiently segregate the extremes in vigor and weakness necessary to exert any marked influence on canker growth. It was observed, however, that great vigor, as in the case of young, very fast-growing leaders and shoots, did have a noticeably stimulating effect on the rate of canker growth.

Moreover, it was evident from the data from Revelstoke that extreme lack of vigor exerts a decidedly retarding influence on canker growth. The cankers at Revelstoke were marked in April 1928 and measured the last of September 1928 and again at the beginning of October 1929. During the growing season of 1928, covered by the first measurements, the branches on which the cankers occurred were generally in fair condition. During 1929 a large number of the branches were partially or completely killed by the cankers, and the vigor of the remaining branches was greatly reduced. On these latter branches, the average diameter of which was 0.44 inch, the average longitudinal growth for 24 cankers was 2.99 inches from April 22 to September 23, 1928, and 3.14 inches from September 23, 1928, to October 8, 1929.

These values show that the growth for the growing season from April to September 1928, during which the affected branches were in relatively fair condition, almost equaled the growth for the entire year from September 1928 to October 1929. Naturally, growth during the winter or dormant season is much slower than during the growing season. Nevertheless, it represents a considerable fraction of the total annual growth. This aspect will be discussed in the section on winter growth. It is evident, therefore, that the growth of these cankers for the growing season of 1929, during which the vigor of the affected branches was nearly exhausted, must have been considerably below that for the growing season of 1928. There were no differences in climatic conditions between the two seasons to cause this difference in growth. Therefore, the reduced growth rate of the cankers during 1929 can be explained only on the basis of their extremely reduced vigor in that year. The measurements at Revelstoke afforded the only concrete evidence secured in the present study that vigor had any material effect on the growth of the cankers.

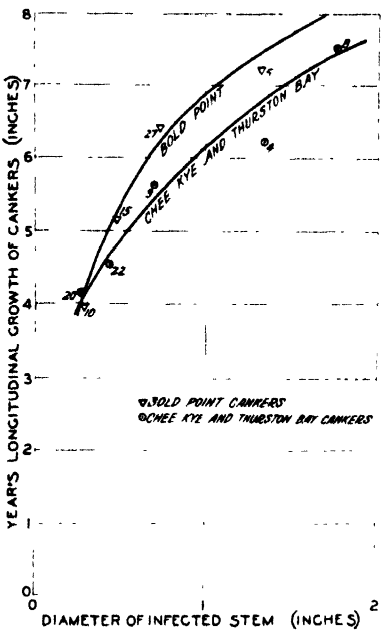

FIGURE 3.—Comparison of year's longitudinal growth of cankers at Chee Kye and Thurston Bay with cankers at Bold Point, for the period from April 1923 to April 1924.

INFLUENCE OF SITE AND CLIMATE

There seems to be a close correlation between regional site conditions and the rate of canker growth. Even when seasonal climatic differences within a given region are slight, the differences in canker growth are frequently marked. During the year 1923–24 the seasonal differences at Bold Point, Chee Kye, and Thurston Bay were too insignificant to become apparent through general observations. All these points lie at low elevations close to the Strait of Georgia and are within the same climatic belt. However, a comparison of canker growth at Bold Point with that at Chee Kye and at Thurston Bay (table 1 and fig. 3) shows that in general the growth of the Bold Point cankers averaged almost one half inch more for the year than that of the cankers in similar stem-diameter classes at Chee Kye and Thurston Bay.

Where the difference in climatic and environmental conditions is greater, the difference in canker-growth rates is striking. This is shown by comparing the growth averages of the cankers measured at Daisy Lake, which has a shorter growing season (table 4), with those of the cankers measured during the same periods at Chee Kye and Thurston Bay (tables 1 and 2). In figure 4 these data are compared graphically.

TABLE 4.—*Average longitudinal growth of branch cankers from April 1923 to April 1924 and of trunk cankers from October 1925 to October 1926, at Daisy Lake, British Columbia*

Stem-diameter class (inches)	Branch cankers marked Apr. 10–16, 1923, measured Apr. 19, 1924			Trunk cankers marked Oct. 31, 1925; measured Oct. 16, 1926		
	Number	Average branch diameter	Canker growth	Number	Average trunk diameter	Canker growth
		Inches	*Inches*		*Inches*	*Inches*
0.1	38	0.134	1.83			
0.2–0.3	37	.216	2.28			
0.4–0.5	1	.500	3.70			
0.6–1.0				[a] 2	0.70	4.25
1.1–1.5				1	1.20	4.40
1.6–2.0				2	1.75	5.35
2.1–2.5				2	2.30	5.55
2.6–3.0				6	2.83	5.60
3.1–4.0				12	3.68	5.93
4.1–5.0				6	4.77	6.85
5.1–6.0				2	5.75	6.80
6.1–7.0				1	6.10	6.60

[a] Marked April 1923; measured April 1924.

Although the growth rates at Daisy Lake were uniformly much below those in the optimum areas, figure 4 shows a similarity in the general form of the curves for the two sets of data. Under conditions represented at Daisy Lake, canker growth increased rapidly from a rate of less than 2 inches per year for cankers in the 0.1-inch stem-diameter class to over 5 inches per year for those with stem diameters of between 1.6 and 2.0 inches. Beyond such diameters the curve of growth rapidly levels off and attains a maximum average of between 6 and 7 inches per year at stem diameters beyond 4 or 5 inches. In

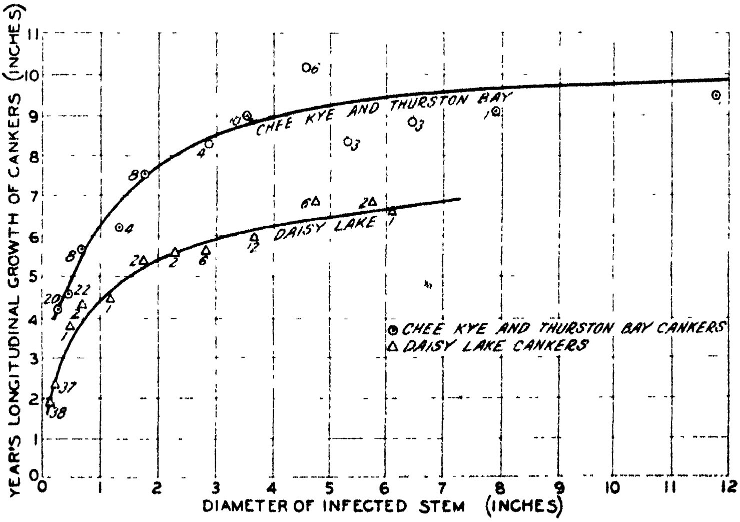

FIGURE 4.—Comparison of year's longitudinal canker-growth rates at Daisy Lake with those at Chee Kye and Thurston Bay.

the optimum areas, on the other hand, the rate increases rapidly from about 4 inches per year in the 0.2- to 0.3-inch stem-diameter class to about 8 inches per year at a stem diameter of about 2 inches. Beyond diameters of 4 or 5 inches the curve of growth levels off to a maximum of between 9 and 10 inches per year.

The similarity in the general climatic conditions of Revelstoke, Arrow Park, and Daisy Lake results in a similarity of canker growth at all three stations. But since only branch cankers were measured at Revelstoke and Arrow Park, no comparison is available in the larger stem-diameter classes; and, as stated previously, the cankers measured at Revelstoke were older than those measured elsewhere and the branches on which they occurred were exceptionally low in vigor, whereas the cankers measured at Arrow Park and the branches on which they occurred were decidedly thrifty; the branches on which cankers were measured at Daisy Lake, on the other hand, were generally in only fair condition. Furthermore, a different annual-growth period was covered at each of the three places. Consequently, the relationships between these areas are somewhat obscure. Nevertheless they afford some significant comparisons with the other data.

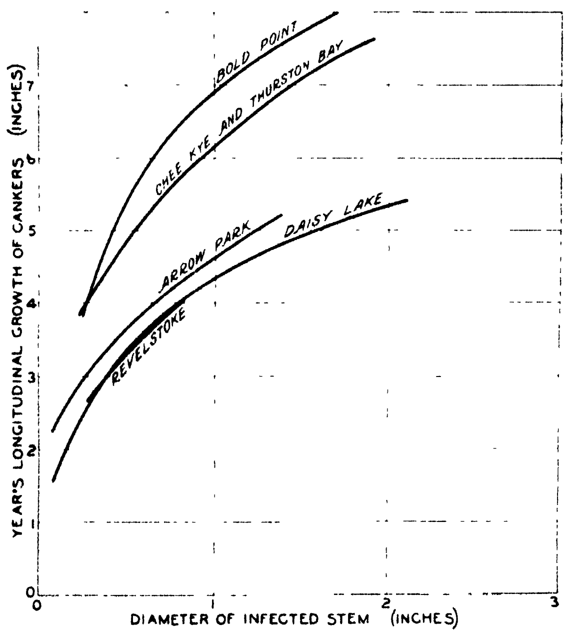

FIGURE 5.—Comparison of year's longitudinal canker-growth rates in all areas.

The longitudinal growth values for the cankers of Revelstoke, Arrow Park, Daisy Lake, Chee Kye and Thurston Bay, and Bold Point are given in table 5. Figure 5 gives the growth curves for all the areas, covering the diameter classes in which all may be compared. The relative thrift conditions of the branches at Arrow Park were entirely comparable with those of Chee Kye and Thurston Bay and those of Bold Point, that is, the branches in each area were generally thrifty for the site conditions represented. The values and curves for Arrow Park, therefore, may be compared with those of the optimum areas to show further evidence of the influence of climate and general site conditions on canker growth. This comparison shows that despite the highly vigorous condition of the affected branches, growth—as in the case of the cankers at Daisy Lake—is decidedly slower than in the optimum areas.

TABLE 5.—*Average annual longitudinal growth of marked cankers in various localities*

Stem-diameter class (inches)	Revelstoke (Sept. 23, 1928, to Oct 8, 1929)			Arrow Park (Oct. 11, 1929, to Sept. 21, 1930)			Daisy Lake			Chee Kye and Thurston Bay			Bold Point (April 1923 to April 1924)		
	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth
	No	In	In	No	In.	In	No.	In.	In.	No.	In.	In.	No.	In.	In.
0.1				7	0.10	2.41	[a]38	0.134	1.83						
0.2-0.3	7	0.29	2.91	112	.23	2.81	[a]37	.216	2.28	[b]20	0.27	4.12	10	0.28	3.93
0.4-0.5	13	.44	2.97	16	.44	3.49	[a]1	.50	3.70	[b]22	.43	4.52	15	.47	5.13
0.6-1.0	4	.73	4.08	2	.65	3.50	[a]2	.70	4.25	[b]9	.69	5.62	27	.73	6.39
1.1-1.5				1	1.20	5.70	[a]1	1.20	4.40	[b]4	1.33	6.20	5	1.32	7.20
1.6-2.0							[c]2	1.75	5.35	[b]8	1.76	7.50			
2.1-2.5							[c]2	2.30	5.55						
2.6-3.0							[c]6	2.83	5.60	[b]2 [d]2	2.88	8.30			
3.1-4.0							[c]12	3.68	5.93	[d]10	3.56	8.98			
4.1-5.0							[c]6	4.77	6.85	[d]6	4.60	10.15			
5.1-6.0							[c]2	5.75	6.80	[d]3	5.33	8.37			
6.1-7.0							[c]1	6.10	6.60	[d]3	6.47	8.83			
7.1-8.0										[d]1	7.90	9.10			
11.1-12.0										[d]1	11.80	9.50			

[a] April 1923 to April 1924

[b] Chee Kye and Thurston Bay (April 1923 to April 1924).

[c] October 1925 to October 1926.

[d] Chee Kye (October 1925 to October 1926).

In connection with the general application of the results reported here the difference shown between the Arrow Park values and those of the optimum areas is important. The Arrow Park area is situated on the western edge of the northward extension of the main commercial range of *Pinus monticola* in the "Inland Empire" section of northern Idaho and adjacent Washington and Montana. The general site and climatic conditions for *P. monticola* in this area are more typical of the main range than are those in the optimum areas.[6] In the main range of the pine, therefore, the rates of canker growth may be expected to fall between that at Arrow Park and the optimum and to be probably somewhat closer to the former than to the latter.

The growth rates at Revelstoke (fig. 5) are among the slowest shown. In this case, however, as has already been shown, the growth of the cankers was materially retarded by the extreme lack of vigor of the affected branches.

WINTER GROWTH

As previously stated, canker growth during the winter, or dormant season, is much slower than during the growing season. In the present studies the growing season is defined as the 6 months following the beginning of general growth activity in the spring, and the winter season as the remaining 6 months of the year. Measurements of the winter growth of cankers were secured at Chee Kye and Thurston Bay in 1924, at Daisy Lake in 1926, and at Arrow Park in 1930. These data, together with those on annual growth for the same cankers are given in table 6. The periods covered in the measurements of winter growth were October 8, 1923, to April 17, 1924, at Chee Kye; October 19, 1923, to April 8, 1924, at Thurston Bay; October 31, 1925, to April 15, 1926, at Daisy Lake; and October 9 to 11, 1929, to May 6, 1930, at Arrow Park.

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[6] The fact that the main commercial range does not include all optimum growth of *Pinus monticola* will undoubtedly seem strange. The reason for this appears primarily to be that in the coastal region of the range of the pine—that is, in the country surrounding the Strait of Georgia and southward, which presents optimum conditions for this species and includes the optimum areas of the present study—the conditions are particularly favorable for its principal bark beetle, *Dendroctonus monticolae* Hopk. Between diameters, at breast height, of about 10 to 20 inches, the pine appears to be especially susceptible to the attacks of this beetle which decimates the stand to a very small and scattered representation at maturity. This has been a common observation of western entomologists. The relationship was first pointed out to the writer in 1928 by Ralph Hopping, Dominion entomologist, at Vernon, British Columbia.

TABLE 6.—*Average annual and winter longitudinal growth of cankers at various localities*

Stem-diameter class (inches)	Chee Kye and Thurston Bay					Daisy Lake					Arrow Park				
	Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth		
			Annual	Winter				Annual	Winter				Annual	Winter	
	No.	*In*	*In*	*In*	*Pct* [a]	*No*	*In*	*In*	*In*	*Pct* [a]	*No*	*In*	*In*	*In*	*Pct* [a]
0.1											7	0.10	2.41	0.40	16.6
0.2-0.3	20	0.28	4.12	0.68	16.5						112	.23	2.81	.46	16.4
0.4-0.5	[b]21	.43	4.50	.84	18.7						16	.44	3.49	.73	20.9
0.6-1.0	9	.69	5.62	.94	16.7						2	.65	3.50	.55	15.7
1.1-1.5	4	1.33	6.20	.98	15.8	1	1.20	4.40	0.9	20.5	1	1.20	5.70	1.30	22.8
1.6-2.0	8	1.76	7.50	1.08	14.4	2	1.75	5.35	1.0	18.7					
2.1-2.5						2	2.30	5.55	.8	14.4					
2.6-3.0	2	2.90	8.45	1.20	14.2	6	2.83	5.60	.65	11.6					
3.1-4.0						12	3.68	5.93	.90	15.2					
4.1-5.0						5	4.76	6.42	1.22	19.0					
5.1-6.0						2	5.75	6.80	.85	12.5					
6.1-7.0						1	6.10	6.60	.60	9.1					
Total or av.	64	.72	5.14	.85	16.5	31	3.61	5.91	.89	15.1	138	.26	2.90	.50	17.2

[a] Winter growth expressed as percentage of annual growth.

[b] 1 canker given in table 5 not included for lack of separate winter growth measurements.

The winter growth averages about 15 to 17 percent of the annual growth, and about 18 to 20 percent, or about one sixth to one fifth, of that made during the growing season. There was considerable variation in the winter and the summer growth of individual cankers, although the total or annual growth values in these cases were fairly close to the averages. This fact indicates a rather wide variation in the periods of main growth activity for individual cankers, as was brought out by Rhoads' studies in *Pinus strobus* in 1918 (*6, p. 517*).

UPWARD AND DOWNWARD GROWTH

Throughout the present studies, measurements were taken of the longitudinal extensions of the cankers in both directions, that is, upward, or against the sap stream in the bark of the branch or trunk, and downward, or with the sap stream. The measurements generally showed a greater growth downward than upward. The same tendency was noted on *Pinus strobus* by Rhoads in 1918 (*6, p. 516*). Downward and upward growth averages for the main canker groups in the present study are given in table 7.

TABLE 7.—*Average annual downward and upward growth of cankers in various localities*

Locality	Type of cankers	Year of measurement	Cankers	Average stem diameter	Canker growth				
					Average total	Average upward	Downward		
							Average	Proportion of total growth	Superiority over upward growth
			Number	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*
Bold Point	Branch	1924	57	0.63	5.70	2.71	2.98	52.4	10.0
Chee Kye and Thurston Bay.	do	1924	47	.39	4.48	2.05	2.43	54.3	18.9
Do	Trunk	1924	19	1.67	6.88	3.31	3.57	51.9	7.9
Daisy Lake	Branch	1924	76	.18	2.08	.89	1.19	57.2	33.6
Do	Trunk	1926	32	3.64	6.01	3.03	2.98	49.5	[a] 2.0
Chee Kye	do	1926	26	4.77	8.97	4.48	4.49	50.0	2
Revelstoke	Branch	1929	24	.44	3.14	1.28	1.85	59.1	44.5
Arrow Park	do	1930	138	.26	2.90	1.37	1.53	52.8	12.1
Total			419						

[a] Inferiority.

The superiority of downward growth over upward growth appears to be very largely due to the size of the part infected, since downward growth runs into greater stem diameters, whereas upward growth runs into smaller diameters. As might be expected on this basis, the difference between downward and upward growth is generally greater on the twigs and branches than on the trunks, since relative taper is normally more rapid in the twigs and branches than in the trunks. Furthermore, the influence of the difference in diameters at the lower and upper ends of the canker is accentuated in the branches because of the fact that the branches fall in the smaller diameter classes where differences in stem diameter have the greatest effect on canker growth. The difference between downward and upward growth is particularly marked in the case of the Daisy Lake branch cankers, where growth of the affected branches was slower and taper was more rapid than normally. The difference was still more marked in the Revelstoke branch cankers, which, being older and therefore relatively longer than the average, extended over greater lengths of the branches and thereby increased the differences in stem diameter at the lower and upper ends of the cankers, from which growth was measured.

In addition to the influence of differences in diameter, the superiority in downward growth may be partly accounted for on the hypothesis that downward growth of the mycelium is aided by the downward flow of sap in the bark. There was, however, no means of checking such an assumption.

While downward growth was generally greater than upward growth, there were frequent individual exceptions to this rule, and sometimes upward growth was very decidedly greater than downward growth. It was frequently observed that longitudinal growth in both directions was checked for a time at the nodes, particularly where branching was heavy and the nodes were enlarged and swollen. In most cases where the upward growth was the greater, the downward growth had been retarded from this cause. In some cases, however, where such an explanation was not applicable, the cause of the excess in upward growth could not be determined.

LATERAL OR GIRDLING GROWTH

Equally as important as longitudinal growth, or even more important, is the growth of the canker laterally, since it is through growth in this direction that the stem is finally girdled and killed. Measurements on such growth were secured wherever possible in all cankers studied. In the case of the cankers which were marked on the branches, the infection had generally completely surrounded the stem at the time of marking. Therefore trunk cankers, and mainly the larger ones, provided the chief basis for measurements of lateral growth. The data secured for annual growth in this direction were practically confined to the 1926 trunk-canker measurements at Chee Kye and Thurston Bay and at Daisy Lake. Table 8 gives annual longitudinal and lateral growth averages of all cankers on which complete measurements of both longitudinal and lateral growth were secured. No difference was noted, or expected, in the average rates of extension to the right and left, and the values are therefore combined in table 8.

TABLE 8. —*Average annual growth of cankers at Chee Kye and Daisy Lake, British Columbia*

Stem-diameter class (inches)	Chee Kye, Oct. 20-21, 1925, to Oct. 13-14, 1926					Daisy Lake, Oct. 31, 1925, to Oct. 16, 1926				
	Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth		
			Longitudinal	Lateral				Longitudinal	Lateral	
	Number	*Inches*	*Inches*	*Inches*	*Percent* [a]	*Number*	*Inches*	*Inches*	*Inches*	*Percent* [a]
1.6-2.0		----	----	--	----	1	1.60	5.50	2.00	36.4
2.1-2.5		-	----	--	--	2	2.30	5.55	2.15	38.7
2.6-3.0	2	2.85	8.15	2.50	30.7	5	2.84	5.54	2.52	45.5
3.1-4.0	9	3.60	8.88	3.21	36.1	12	3.68	5.93	2.61	44.0
4.1-5.0	4	4.60	9.45	3.48	36.8	6	4.77	6.85	2.55	37.2
5.1-6.0	3	5.33	8.37	3.67	43.8	2	5.75	6.80	2.65	39.0
6.1-7.0	2	6.60	8.70	3.65	42.0	1	6.10	6.60	2.40	36.4
11.1-12.0	1	11.80	9.50	3.70	38.5		---	---		--
Total or average	21	4.64	8.86	3.33	37.6	29	3.82	6.10	2.52	41.3

[a] Lateral growth expressed as percentage of longitudinal growth.

The data in table 8 show that lateral growth is decidedly slower than longitudinal growth. In general, it amounts to about 40 percent of the longitudinal growth. In these proportions lateral growth appears to show about the same variations as longitudinal growth with respect to all the influences affecting the latter.

One rather singular exception to the general relations between lateral and longitudinal growth is exhibited in the earliest stages of the young canker, particularly on the larger stems. For perhaps the first 2 or 3 weeks following the appearance of the canker, the discoloration advances so much more rapidly laterally than longitudinally that in this stage the initial discoloration assumes an oval shape, having the long diameter at right angles to the longitudinal axis of the stem. Later the longitudinal growth is accelerated to its normal rate and the discoloration assumes its characteristic shape, that is, an oval having the long diameter parallel with the longitudinal axis of the stem. This phenomenon would be an interesting subject for physiological study.

The foregoing data have dealt almost entirely with averages. In some cases the variations from these averages were rather wide, but in general the individual measurements were fairly close to the averages. Because of this fact and in view of the consistent relations shown by data from different localities and sets of cankers, despite the frequently small bases, a statistical presentation of means and deviations is not considered necessary.

AREAS OF PRODUCTION OF FRUITING STAGES

In connection with the growth measurements, observations were made on the extent and extension of the areas of the canker surface bearing aecia and pycnia. In general, the pycnial zone extends longitudinally to within one half to 3 inches and laterally to within one fourth to 1 inch of the outer limits of the discoloration, depending on the size of the canker and the time of year it is observed. The differences are naturally smaller on the smaller, slower growing cankers. As might be expected, the differences are generally greatest just before pycnia formation begins in summer, for from the beginning of canker growth in the spring the discoloration has been extending while pycnial activity has been quiescent. Thus, the differences are generally least at the height of pycnial activity.

Aecia are produced in the spring on the area which produced pycnia the preceding year. Part of this area may have produced pycnia for the first time 2 or 3 years earlier, and aecia for the first time 1 or 2 years earlier. The production of aecia from the same bark for 2 successive years is the usual occurrence in western white pine. On large stems it frequently occurs for 3 years in succession, and sometimes for 4 years or even more.

In this latter respect the rust considerably exceeds its performance described for *Pinus strobus* (*6*, *p. 515*; *7*, *p. 29*), probably because of the thicker bark and greater food supply for the fungus in *P. monticola*. The aecial zone on *P. monticola* generally extends fairly close to the limits of the preceding year's pycnial zone and may coincide with it. Rhoads (*6*, *p. 515*) has recorded the production of aecia in the discolored area beyond the limits of the pycnial zone on *P. strobus*. The writer does not recollect having observed such an occurrence on *P. monticola*. Very frequently in this species, particularly when the canker is young and pycnia were produced for the first time the preceding year, aecia are not produced at all on the preceding year's pycnial zone, and sometimes the canker may have produced pycnia for 2 years or even more without producing aecia. Normally, however, when the aecia are not produced in the year following that in which the canker bears its first pycnia, they are produced the next spring (*5*).

INJURIOUS EFFECTS OF CANKERS

RELATION TO CANKER GROWTH

Most of the serious injury and killing result from mycelial activity on the trunk so far down from the tip that the death of a large section of the top or of the entire tree ensues. At such points the girdling growth is of chief importance. The higher on the trunk the infection is located, the greater importance downward growth assumes.

The rust infects its aecial hosts through the needles (*1*). In *Pinus monticola* needles are generally present only on the last few internodes of the trunk and branches. In its relation to injury, therefore, the infection of the trunk directly through the needles is relatively unimportant. Infection of the trunk occurs to some extent also through short shoots, the so-called "kurtztriebe" (*8, p. 33*), but although these are not uncommon in *P. monticola*, their insignificance in relation to the whole crown makes them comparatively unimportant as a source of infection. The effect of the fungus on its host depends to a large degree on the specific portion of the crown in which it grows. Serious injury and finally death are caused by eliminating from the life processes of the tree so large a part of the crown as to make further functioning impossible. The invasion of the trunk at a point at which the resulting girdling must end in the killing of a considerable part of the crown is accomplished through the centripetal growth of the mycelium downward from the point of infection in the branch (*7, p. 28*). In the branches, therefore, the downward growth of the canker is of first importance and lateral growth is relatively unimportant, as far as the life of the tree is concerned.

An exception to this general rule occurs in *Pinus monticola* when the infection is so heavy and the branch cankers are so numerous as to seriously damage or kill the tree through killing the branches. Such infection and killing are relatively uncommon, however, and are usually restricted to trees immediately adjacent to the infecting *Ribes* plants or close to *Ribes* concentrations, particularly when the species involved are of high susceptibility. Where large numbers of highly susceptible bushes occur in the stand, such killing of pines may become general in trees up to large pole size. Larger trees are less susceptible to killing in this manner, and in overmature veterans it has not been observed to occur at all. Veteran trees are found in many of the centers of oldest and heaviest infection in British Columbia. These trees have persisted through infection which has already wiped out the younger trees beneath them, and apparently the only manner in which such trees are killed or seriously injured is by spread of the fungus down the branches and into the bole.

The average rates of lateral and downward growths of cankers are compared in table 9, in which the Chee Kye and Thurston Bay canker measurements of 1924 and 1926 represent the optimum, and the Daisy Lake canker measurements of 1924 and 1926 represent slower growing conditions. These data are shown as curves in figure 6.

TABLE 9 —*Average annual downward and lateral canker growth rates as represented by measurements in 1924 and 1926 at typical areas in British Columbia*

Stem diameter class (inches)	Chee Kye and Thurston Bay						Daisy Lake					
	Downward growth			Lateral growth			Downward growth			Lateral growth		
	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth
	Number	*In*	*In*	*Number*	*In*	*In*	*Number*	*In*	*In*	*Number*	*In*	*In*
0 1							48	0 13	1 05			
0 2-0 3	20	0 28	2 20				37	22	1 30			
0 4 0 5	22	44	2 47				1	50	2 20			
0 6 1 0	10	69	3 05				2	70	2 35			
1 1 1 5	4	1 33	3 25	1	1 30	2 80	1	1 20	2 00			
1 6-2 0	8	1 76	3 79				2	1 75	2 70	1	1 60	2 00
2 1-2 5	2	2 50	3 20				2	2 40	2 45	2	2 30	2 15
2 6-3 0	4	2 88	4 05	2	2 85	2 75	6	2 83	2 72	5	2 84	2 52
3 1 4 0	12	3 56	4 46	9	3 60	3 21	13	3 66	2 91	14	3 64	2 59
4 1 5 0	6	4 40	4 70	[illegible]	4 68	3 44	6	4 77	3 57	6	4 77	2 55
5 1 6 0	3	5 33	4 20	4	5 48	3 48	3	5 53	3 33	3	5 53	2 70
6 1 7 0	4	6 45	4 80	4	6 60	3 80	1	6 10	3 60	1	6 10	2 40
7 1 8 0	1	7 90	4 60									
8 1 9 0										1	8 20	2 70
11 1 12 0	1	11 80	5 50	1	11 80	3 70						

Table 9 and figure 6 show that the averages of downward growth in the optimum areas range from a minimum of about 2 inches a year in the smallest diameter classes to a maximum of about 5 inches a year in the largest diameter classes In the slower growing areas, the averages range from a minimum of about 1 inch per year to a maximum of about 3 5 inches per year.

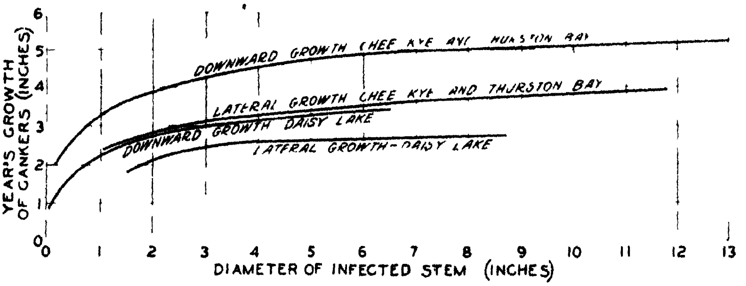

FIGURE 6 Year s downward and lateral growth rates of cankers

Lateral-growth values are confined primarily to diameters ranging between 2 5 and 7 inches for the optimum areas and between 2 and 6 inches for the areas of slower growth. Within these diameter limits the growth averages range in the former areas from about 3 to between 3.5 and 4 inches a year, and in the latter from about 2.3 inches to between 2.5 and 3 inches a year.

Figure 6 shows that the curves for downward growth have the same general form as those for total longitudinal growth (figs 2 and 4) In the smaller diameter classes the averages rise sharply to nearly 4 inches per year for diameters of 2 inches in the optimum areas and to over 2 inches per year for diameters of 1 inch in the slower growing areas. Beyond these diameters they rise more slowly and level off to a more or less constant maximum of about 5 inches a year for diameters above 6 inches in the former areas and to a maximum of around 3.5 inches per year for diameters above 5 inches in the latter areas.

No data exist for lateral growth rates in the smaller diameters. The curves for lateral growth tend to assume, in the sections in which they may be compared with the curves for longitudinal growth, a form similar to the curves for downward growth, and it is probable that an extension of them on this basis into the smaller diameter classes would fairly accurately represent actual lateral growth values for these classes.

Since the distance to be covered peripherally is short in the smaller twigs and branches, these are girdled very rapidly. In twigs and branches having diameters approaching 1 inch, girdling generally occurs within a year after the canker makes its appearance. Beyond this diameter the time required for girdling is longer. In stems over 3 inches in diameter, the number of years required for girdling is in general roughly equal to the number of inches of diameter.

In the smallest twigs the portion of the twig beyond the canker is usually killed within 1 or 2 years following girdling, or within 2 or 3 years following the appearance of the canker in this class of stems. The death of the portion of the stem beyond the canker is termed "flag formation" because of the conspicuous red-brown color of the dead foliage of the affected part. In the intermediate branches and smaller trunks, this killing of the part beyond the canker, or flag formation, usually occurs within 2 or 3 years following girdling, but in some cases it may require as much as 4 years or more. In the larger trunks it generally occurs within 1 to 3 years. The greater weakening which occurs during the longer period required for girdling large trunks accounts for the greater rapidity with which death follows such girdling.

Death of the stem below the canker does not occur at the same time as the death of the portion beyond, except where the canker is located so far down as to kill the entire branch, or the entire tree in the case of infection on the trunk. Where the stem below the canker is not killed, downward growth of the fungus continues for an indefinite period depending on the number, thrift, and disposition of the feeding branches below, with further die-back following irregularly behind its downward advance. If the branches below are sufficiently numerous, vigorous, and closely disposed, the fungus will continue its downward growth until, in the case of infection on the branches, it reaches the bole; or until, in the case of infection of the trunk, it kills the tree.

Where the lower limits of the canker have progressed for a considerable distance down an internode, it is a common occurrence for the die-back to include the upper portion of the internode. The lower portion with the living remains of the canker at its upper end is thus left without any source of food supply from above. In these cases, despite this condition, the lower portion of the internode regularly remains alive for some time, permitting the fungus to continue its spread toward the region of the living branches in the next whorl below.

Investigations not yet reported, in which cankered branches were cut off with a knife near the lower limits of the canker, and uncankered branches were cut to form similar uninfected stubs, suggested that the presence of the infection at the end of the stub had the effect of stimulating a reversal of flow of the elaborated food materials. The cankered stubs remained alive, and downward growth of the infection on them continued for periods ranging up to several years, while the uncankered stubs almost invariably died within a few months.

In flag formation there frequently appears to be a partial decline of the stem for some distance below the canker, and the portion of the internode remaining below the die-back usually does not continue to live as long as did the cankered stubs. It generally remains alive for a full growing season, however, and commonly for as much as 2 years.

Despite the factors favoring its downward progress, the canker very often encounters an internode over which it is unable to pass, and the infection dies out. This is a particularly frequent occurrence in the smaller trunks or in the tops of the larger trees. The internodes of the trunks are normally longer than elsewhere in the tree, and where the diameter is small enough for girdling to occur within a few years, the fungus is unable to make sufficient downward progress, by the time the portion of the leader above the point of girdling dies, to traverse the remaining portion of the internode before the latter also succumbs. In portions of the trunks having larger diameters, where greater periods are required for girdling, internodes of similar length may be traversed easily.

In the smallest twigs and branches, internodes greater than 10 inches in length will generally halt the downward advance of the canker. In branches one half to 1 inch in diameter the infection generally dies out on internodes greater than 12 to 15 inches in length. On the larger branches still greater lengths of internode may be traversed; but since, where the stem is large, most of the foliage of the branch is beyond the canker, the entire branch is likely to die at the time of flag formation. Frequently the inner side branches have been partially or completely killed by shade, resulting in a length of partly or entirely unsupported stem over which the infection would be incapable of passing under any circumstances. This is particularly the case in the lower crown, where the inner branches have undergone the most intense shading and where all the branches are generally under suppression. On suppressed branches, even though the canker has not yet reached an internode that it cannot pass over, the whole branch may be killed at flag formation or within a short time afterward because of the combined influence of the canker and of suppression. Where the branch has few feeders or is otherwise in poor condition, it may likewise be killed within a short time; or it may be killed primarily from suppression or from some other cause aside from blister rust. When the branch or any part of it is killed from any cause, all the cankers on it or its affected portions are killed at the same time. Thus a large proportion of the individual infections are eliminated before they can reach the trunk.

Where the die-back is only partial and the mycelium is not completely killed, the rate of downward growth is variable. When the portion of the branch below the infection remains vigorous, the growth is entirely comparable to that on stems of similar size and condition in which no die-back has occurred. As already stated, at the time of flag formation and for some time previous there is a tendency toward decline in the portions of the branch below the canker as well as above it. Measurements of cankers on the branches which remained entirely alive for the year following marking at Revelstoke in April 1928 (p. 482) showed that this loss of vigor may occur to such an extent as to retard the growth of the fungus. These measurements included only a portion of the cankers originally marked. In the remainder, complete longitudinal measurements were prevented either by flag formation or death of the entire branch. Where flag formation occurred, however, measurements were continued of the downward growth of the cankers. These measurements are the only values secured on downward growth following flag formation. They are arranged by stem-diameter classes in table 10. Similarly arranged measurements of cankers on branches which remained entirely alive are included for comparison.

Table 10.—*Average downward growth from Apr. 22, 1928, to Oct. 8, 1929, of cankers on branches partly destroyed by flag formation in 1928 and 1929, and on branches that remained entirely alive during the same period, at Revelstoke, British Columbia*

Stem-diameter class (inches)	Condition of affected branch														
	Portion beyond canker died during 1928 growing season					Portion beyond canker died during 1929 growing season					Entire branch remained alive				
	Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth		
			Apr. 22 to Sept 23, 1928	Sept 23, 1928, to Oct 8, 1929	1928 growth as percentage of 1929 growth			Apr 22 to Sept 23, 1928	Sept 23, 1928, to Oct 9, 1929	1928 growth as percentage of 1929 growth			Apr. 22 to Sept 23, 1928	Sept. 23, 1928, to Oct 8, 1929	1928 growth as percentage of 1929 growth
	No	*In*	*In*	*In*	*Pct.*	*No*	*In*	*In*	*In*	*Pct*	*No*	*In*	*In*	*In*	*Pct*
0.2-0.3	4	0.20	1.23	1.23	100.0	17	0.26	1.38	1.49	92.6	7	0.29	1.34	1.66	80.7
0.4-0.5	1	.40	2.20	2.40	91.7	5	.46	1.82	1.70	107.1	13	.44	1.78	1.77	100.9
0.5-1.0		--	---	--	-----	4	.65	1.98	2.40	82.5	4	.73	1.93	2.48	77.8

Table 10 shows that downward growth has continued at rates fairly comparable to those maintained before flag formation. In the 0.2- to 0.3-inch diameter class, 1929 growth, as compared with 1928 growth, is relatively slower for cankers in which flag formation occurred in 1928 than for cankers in which flag formation occurred in 1929 or in the cases in which the entire branch remained alive. In the 0.4- to 0.5-inch diameter class this value is higher, but only a single canker is represented. The cankers in which flag formation occurred in 1929 show a proportionally slower growth in 1929 compared with 1928 than the cankers on branches that remained alive. As has already been shown, the 1929 growth of these latter cankers was relatively slower than their growth in 1928 because of the exhausted condition of the branches on which they occurred. Evidently the branches having cankers in which flag formation occurred were generally even more exhausted.

In the cankers in which flag formation occurred in 1929, where there is a fair basis in number of cankers, a very wide variation in

1929 growth as compared with 1928 was evident. In some cases it was far below 1928 growth, in others far above. This is shown in table 11, which gives individually the comparative 1928 and 1929 growths for these cankers and for those on branches that remained entirely alive.

TABLE 11.—*Downward growth of individual cankers in 1928 and 1929 on branches partly destroyed by flag formation in 1929 and on branches that remained entirely alive during 1929, at Revelstoke, British Columbia*

Branch beyond canker died during growing season of 1929			Branch remained entirely alive in 1929		
Stem diameter (inches)	Canker growth		Stem diameter (inches)	Canker growth	
	1928	1929		1928	1929
	Inches	*Inches*		*Inches*	*Inches*
0.2	0.7	1.0	0.2	1.3	1.6
	1.5	1.0	.3	1.6	2.8
	1.5	1.4		1.5	1.5
	1.2	1.1		1.1	1.2
	1.2	1.5		1.6	1.3
	1.6	.4		.9	1.3
.3	1.6	1.8		1.4	1.9
	1.8	1.2	.4	1.8	2.0
	1.3	.9		1.7	1.6
	1.3	4.3		1.7	1.6
	1.9	.8		1.7	1.6
	1.2	1.8		1.7	1.5
	1.3	1.8		1.5	1.6
	1.7	.7		2.0	1.3
	1.7	2.1		2.2	1.9
	1.1	1.6	.5	1.8	1.5
	.8	1.9		2.1	2.4
.4	2.1	1.2		1.4	2.0
	1.9	1.7		1.2	2.0
.5	1.9	2.2		2.4	2.0
	1.6	1.5	.7	1.7	2.5
	1.6	1.9		2.1	2.7
.6	3.0	1.6		1.7	2.6
	1.5	2.5	.8	2.2	2.1
.7	2.2	3.0			
	1.2	2.5			

Table 11 shows that there was a far greater variation among the cankers in which flag formation occurred than among those on branches that remained alive. Evidently in some of the former the occurrence of flag formation was followed by a stimulation or release from retardation of downward growth. Apparently the sickly condition of the portion of the branch beyond the canker constitutes a decided drain on the healthy portion below the canker. This drain is eliminated when die-back occurs and the outward flow of water into the dying portion is stopped. The remaining portion of the branch recuperates sufficiently to permit downward growth of the fungus again to become normal. This tendency toward recovery following flag formation has been a common observation where the canker was not too far down on the branch and where the condition of the branch was unaffected otherwise than by the canker.

Where the canker occurs too far down on the branch or the branch is under the influence of suppression, a continued decline instead of recovery is the rule. The effect of this decline is apparent in table 11 where the 1929 growth-rate value is below that of 1928.

Although no measurements are available as proof, the writer has observed under certain conditions what appeared to be a decided tendency of downward growth to accelerate with the approach of death in the affected tissues. This tendency was apparent where an entire branch, otherwise unaffected, was being killed by the canker and also where the canker following flag formation had not quite traversed a long internode which was about to succumb and below which the branch was vigorous. This apparent acceleration seems to suggest some inherited physiological reaction of the parasite which would bring the mycelium in contact with a better source of food supply and thus perpetuate the life of the organism in the host.

METHOD OF CALCULATING TIME AND MANNER OF INJURY

From the known rates and habits of canker growth, it is possible to calculate and estimate the probable development of the individual infection. This permits, far in advance of the occurrence, calculation and fairly accurate prediction of the time and extent of injury that will result from any given condition of infection on the pines. Special studies, not yet reported, have been made in this connection. This application of the knowledge of canker growth rates and behavior, however, may be briefly illustrated by a hypothetical case, as follows:

A young canker has entered from a side twig on a lower branch of good condition on a vigorous young tree 30 feet high in a moderately dense stand in the optimum pine range. The canker is 3 feet from the trunk. The diameter of the branch below the canker is 0.7 inch, and at its juncture with the bole is 1.0 inch. There are numerous "feeders", or living side branches, below the canker. The internodes are short enough for the canker to grow over. The diameter of the bole at the point of juncture with the branch is 4.0 inches. By referring to the curve of downward growth for optimum areas (fig. 6), it is found that the rate of downward growth on the branch should average about 3 inches a year. At this rate is would take 12 years for the infection to reach the bole. It is necessary next to visualize the probable changes that will take place in the tree during this time. At the present rate of growth it is calculated that the stand will have increased in this time by an average of about 20 feet in height and it is probable that within 5 years the stand will be closed and that this branch will have become suppressed. It is practically certain that the effects of suppression and the canker together will kill the branch and that the canker will consequently die before it can reach the bole.

Branch cankers high up in the upper crown of the same tree might be thought to be relatively innocuous, since so little of the crown is involved above them; but this is not the case. For example, a canker may occur 2 feet out on a branch at 22 feet from the ground, which has sufficient feeders, etc., to permit the canker to grow down to the trunk. The diameter of the branch below the canker is perhaps 0.3 inch, and at its juncture with the bole 0.6 inch. The diameter of the trunk at this point is 1.6 inches. The average annual growth of the trunk is 20 inches in height and, at the point at which the infected branch enters, 0.4 inch in diameter. It is calculated that the downward growth of the canker will average about 2.4 inches per year and that it will require about 10 years for the canker to reach the trunk.

By this time, if the growth in height maintains its present pace—and there is every indication from the growth of older trees that it will—the tree will be about 47 feet high. Its diameter at 22 feet will have increased to about 5.5 inches. It will then require about 5 years for the infection to girdle the tree; during this time growth in height may be retarded by the infection, but will amount to about 7 feet more. During these years the base of the crown has moved up from near ground level to about 15 feet above ground level, owing to suppression of the lowest branches. Thus, at the time of girdling the tree will be about 55 feet high, and the girdling, at 22 feet, will be near the base of the crown. Obviously the tree will be killed.

For comparison with this last case, let it be assumed that on the same branch the infection is located within 10 inches of the bole instead of 2 feet out. The diameter of the branch below the canker is 0.6 inch. The average downward growth rate of the canker is calculated as 2.7 inches per year. In 4 years it will have reached the bole. The diameter of the latter at the point of juncture with the branch will then be about 3.5 inches. It will require about 3 years for tne infection to girdle it. In the 7 years required for growth down the branch and girdling, assuming that growth in height was retarded by the girdling action of the fungus to an average of about 15 inches per year during the last 3 years, the height of the tree will have increased about 11 feet, to a total of about 41 feet. In this case, allowing 2 years for the death of the leader following girdling and 1 more foot of growth in height during this time, about 20 feet of the leader will be lost after the tree has attained a height of about 42 feet, and the tree will be killed or at least so seriously damaged as to be worthless.

Assuming that the infection occurred directly on the stem, about 12 feet of the leader would be killed after the tree had attained about 34 feet in height. This also would be serious, but the tree would probably recover; for it is unlikely that the canker would be able to grow down over the 20-inch internode below it and the infection would die out with the die-back of the leader. In this case a volunteer would take the place of the old leader, and the tree, if free from further infection, might still make a good timber tree.

If the annual growth of the tree were slow in the last two cases, averaging, for example, only 8 instead of 20 inches in height, and 0.2 instead of 0.4 inch in diameter, the sections of the leader killed would be only about 13 feet and 9 feet after the tree had reached the height of about 35 feet and 31 feet, respectively. In these cases, however, the fungus after reaching the bole would easily be able to span the 8-inch internodes below, and the infection would undoubtedly continue its downward spread on the trunk until the tree was destroyed.

It may therefore be concluded that infection at practically any point on the bole will usually kill or render worthless trees of the height class used in the examples or smaller trees.

In large trees approaching or past maturity, infections near the tip are relatively harmless. In mature trees growth in height is generally slower than in trees of the younger classes. Consequently the length of the leader above the canker increases less prior to girdling, and smaller sections of the leader are killed from cankers originating at similar positions in the tops of such trees than in the tops of younger trees. Furthermore, the interference with growth in height is less important in trees of the older classes, because they have already developed to merchantable size; whereas, in the younger classes, continued growth in height is essential to the production of timber. Owing to the much greater length and size of crown in the larger trees, correspondingly greater lengths of the leader may be lost without killing or greatly retarding the growth of such trees. Therefore, since most of the timber volume is in the lower bole, considerable lengths of the leader may be killed in large trees without serious loss in merchantable volume or great reduction in volume increment, whereas in the small trees the infection prevents the production of timber.

In order to kill or cause serious damage in a large tree, infection must generally gain entrance to the bole at a considerable distance below the top. Since the lower branches of the crown are longer and their needles through which infection occurs are consequently farther from the bole, cankers in these branches are normally farther from the bole than are the cankers in the upper branches or in smaller trees. Because of the greater distance to be traversed and the greater chance that the canker will encounter an internode over which it will be unable to pass, and because of the relatively smaller number of feeding side branches close to the bole and the greater chance that the canker will kill the entire branch and itself at the same time, a much smaller proportion of cankers gain entrance to the bole from the older branches than from the younger branches.

Furthermore, the cankers that gain entrance to the bole in the vulnerable portions of the larger trees usually have a greater distance to travel than do the cankers in the younger trees, and the diameter of the bole is of course much greater. Therefore, a greater number of years is generally required for the infection to reach and girdle the bole and to kill or seriously damage the larger trees.

This does not mean that damage will not readily occur in mature trees of large size. Studies indicate that an average of 10 to 20 cankers is required to kill or seriously injure these larger size classes, whereas 1 canker suffices to kill the smallest tree. On the other hand, the much larger crowns of the large trees present a far greater target for the sporidia than do the crowns of the small trees. In spite of the fact that the crowns of the large trees are higher from the ground and consequently more removed and less directly exposed to the sporidia from the infected *Ribes* plants below than are the crowns of the smaller trees, there is a very rapid increase in the total number of cankers with increase in the size of the tree. In general this increase in the total number of cankers more than offsets the difference between the large and small trees in the number of cankers required to kill or seriously injure. The former, therefore, are as much predisposed to killing and serious injury as are the small trees. The injury merely requires a longer period to become effective.

The injury to trees of intermediate size is intermediate between that of the larger and that of the smaller trees. The foregoing examples, however, will suffice to illustrate the general application of the data on the development of cankers in relation to their injurious effects. From these data the amount of injury to any particular tree may be calculated.

Besides the data already given, information was secured on certain phases of canker development which, though of secondary importance with relation to the mode of injury, are of interest in connection with the general description of the cankers. These phases concerned the rate and extent of swelling or constriction.

SWELLING AND CONSTRICTION

The rate of swelling of the cankers, as measured by the increase in the diameter of the stem at the center of the canker, ranged from 0 to 0.3 inch per year, depending on the age of the canker and the size of the part infected. Swelling commenced with the incipient stages of canker formation but did not usually become pronounced until after the discoloration had girdled the stem. As previously mentioned girdling usually occurs within a few months on the smaller branches, whereas on the larger stems it requires a period of years. As the rate of swelling in proportion to the rate of longitudinal growth is most rapid in the younger, smaller stems, the amount of swelling in proportion to the length is greatest in young cankers on such stems. After girdling on the smaller twigs and branches, the canker rapidly develops a short fusiform shape, which is maintained for a time; then the rate of swelling slows down, and as longitudinal growth continues the canker becomes greatly elongated. The greater the size of the stem, the slower is the rate of swelling and the less fusiform is the shape of the canker. On the larger stems the proportion of swelling to length of the canker is slight or negligible and diameter growth of the wood may ultimately be so reduced that the canker assumes the form of a constriction.

Except on the smallest twigs and branches, the bark of the canker generally becomes roughened and cracked in old age. The early death of the part infected usually prevents development to this stage on twigs and small branches. In the larger stems, particularly on the trunks, this cracking of the bark is frequently accompanied by resin flow, which in the case of old cankers on the larger trunks is often heavy.

Constriction, as described by Rhoads (*6, pp. 519-520*), begins with the reduction of the wood increment beneath the infected bark. He found that either retention of normal outline or constriction was the rule in *Pinus strobus* for cankers on stems 2 inches or more in diameter. As a rule, the infected stems retained their normal diameter for a time and became constricted only after the cankers had developed for a long time. Stem analyses showed that although the bark swelled in the earlier stages of the development of the canker, the reduction of wood increment beneath resulted in restoring the normal outline of the twig or in producing a constriction.

In *Pinus monticola* this process is not ordinarily found at such small diameters and the development of noticeable swelling is the rule at stem diameters up to 4 or 5 inches and is frequently found at even larger diameters. In the small twigs and branches there is frequently in the beginning a swelling of the outer xylem elements as well as of the bark, so that after the branch has died and the bark has sloughed off, the portion of the canker developed during the earlier stages of the infection is often still clearly evident as a fusiform swelling of the decorticated stem. Such swelling was apparently not noted by Rhoads in *Pinus strobus*.

During a trip through infection areas of the eastern part of the United States in 1927 the writer was struck by the frequent occurrence of exaggerated constrictions in *Pinus strobus* caused by the rust on trunks ranging mainly from about 2 to 6 or 7 inches in diameter. These trunks had been girdled for some years, and diameter growth at the canker had been completely stopped. The bark tissues appeared to be almost or entirely dead and were largely impregnated with resin. The outer layers of the sapwood also were dead or dying and becoming impregnated with resin, but the inner portions were still alive and functioning. While downward conduction had been almost or entirely blocked, upward conduction had been maintained to such an extent that the leader above had continued to grow almost normally in height and diameter, except for a short distance just above the canker, where the stoppage of downward conduction had resulted in an acceleration of growth in diameter. Below the canker, the growth in diameter had also continued, but generally more slowly. The elaborated food materials for this latter growth had apparently been provided primarily from portions of the crown below the canker. In some cases where most of the infected bark had died, the growth, particularly above the canker, evidenced a distinct attempt at callus formation to heal over the affected portion of the stem. Some of the most exaggerated of these constrictions showed diameters above and below the canker of over twice that of the affected portion. Naturally these constrictions formed points extremely susceptible to breakage, and many trees had been broken at such points by wind and snow.

In *Pinus monticola* formation of such constrictions and resultant breakage has been as yet relatively rare, although breakage is common at the point of the canker in the larger tops and branches after the portions beyond the canker have been killed and rot fungi have found an entrance.

The reason for these differences in canker formation and development in the two species probably lies in the fact that the living bark tissues are generally thicker in *Pinus monticola* than in *P. strobus* and thus provide a food supply that is less readily exhausted by the fungus and consequently a substratum that is less readily killed.

SUMMARY

The growth of over 400 cankers of *Cronartium ribicola* Fischer on *Pinus monticola* Doug. was measured in six different areas in southwestern British Columbia, representing a variety of climatic and environmental conditions for the native range of the host. The cankered stems ranged in diameter from the smallest twigs to trunks up to 8 inches, and in conditions from poor to thrifty.

Size of the infected stem (i.e., twig, branch, or trunk) and regional site conditions were the predominant influences in canker growth rates. Vigor of the infected stem was of relatively little importance.

The curves for longitudinal growth were parabolic in form, rising steeply between stem diameters of 1 to 2 inches, and tending to become almost horizontal at diameters beyond 5 inches.

An average of about 85 percent of the year's growth took place in the cankers from spring to fall.

The cankers grew more rapidly downward than upward, apparently because the diameter of the lower portion of the stem is normally greater than that of the upper portion. On the trunk, which tapers slowly, downward growth of the cankers averaged nearly the same as upward growth; on the smaller, slower growing twigs, which taper rapidly, downward growth was over 30 percent greater than upward growth. In optimum areas the average annual downward growth ranged from about 2 inches on the smallest twigs to about 5 inches on stems more than 6 inches in diameter; in similar stem-diameter classes, in the areas where growth was slower, average downward growth ranged from about 1 inch to between 3½ and 4 inches per year.

Lateral or girdling growth was measured only on trees of the larger stem classes. In trees of the largest stem diameters the average rates of lateral growth ranged to about 3½ inches a year in the optimum areas and to between 2½ and 3 inches in areas of slower growth.

In the smallest stems, girdling occurred within a few months during the growing season. In the larger stems the average number of years required for girdling was about the same as the number of inches of stem diameter.

Aeciospore production continued regularly for several years in succession on the same areas of bark.

The stem is generally killed down to the lower portions of the canker within 1 to 4 years following girdling. If the canker occurs far enough down on the stem, or if the stem is in a weakened condition, the entire branch or trunk may succumb at the time of this first killing or shortly afterward. Otherwise the canker continues its downward growth with further die-back following irregularly behind it. Frequently this progressive die-back takes in the canker itself. In this manner, and also through the death of entire branches owing mainly to the action of the canker and of suppression, great numbers of the cankers die out before they can reach the trunk. The dying out of cankers is greatest on the larger trees where the branches are longer and the lower and inner portions of the crowns are under suppression. On the other hand such trees with their large crowns are more exposed to infection, which in general results in a greater number of cankers.

Most of the serious injury and killing of trees results from girdling well down on the trunk by cankers that have grown down from the branches. Examples are given of the application of the data on canker development to calculations of the time element and the manner of killing or injury under determinable conditions.

Data are presented on canker swelling. The most pronounced swelling occurs on the smallest stems and on these only during the early stages of canker development. In stems over 5 inches the occurrence of swelling is negligible, or constriction develops.

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RUN-OFF AND EROSION FROM PLOTS OF DIFFERENT LENGTHS[1]

By F. L. DULEY, *professor of soils*, and F. G. ACKERMAN, *foreman of farm, Kansas Agricultural Experiment Station*

INTRODUCTION

It has been a common observation among farmers that erosion seems to be more serious on long slopes than on short ones. Probably it has also been assumed that the amount of run-off is greater on long slopes, although less attention has been given to this phase of the subject. As a matter of fact, little definite information is available on these points.

Probably the first tests to determine the effect of length of slope on run-off and erosion were made by Bartel.[2] Work is now in progress at a number of Federal erosion stations, and the preliminary results have been included in the annual reports of these stations. A brief summary of the results has been reported by Middleton and Byers,[3] and Bennett.[4] A study of these results reveals no very consistent trend in the rate of erosion or run-off from slopes of different lengths. Because of this apparent lack of consistency and the consequent need for more definite knowledge, the present work was started. By using methods whereby all factors could be more carefully controlled it was thought that more definite results might be obtained, even though the plots used were smaller than those employed in other tests.

PLAN OF THE EXPERIMENTS

Two sets of experiments were made. In each, 4 plots 3 feet wide and 10, 20, 40, and 100 feet long were used (fig. 1). These were surrounded by strips of galvanized iron set in the ground to a depth of 6 inches. There was a 3-foot alley between plots, and the middle of all plots was on a line at right angles to the direction of the length of the plots. By this arrangement the soil conditions in each plot should be representative of those prevailing over the whole of the small area employed in the experiments. The slope of the land was 4 percent in experiment 1 and 4.4 percent in experiment 2. The plots used in the first experiment were only about 25 feet south of those used in the second.

The soil in which the tests were carried on is Derby silty clay loam. This soil has a fairly permeable surface soil and the subsoil is a silty clay to clay in the B horizon, but has not developed anything that even approaches a clay pan. The soil and subsoil could, therefore, be considered as having characteristics that would permit rather rapid absorption. The surface of the soil was kept free from vegeta-

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[1] Received for publication Oct. 17, 1933; issued June, 1934. Contribution No. 228, Department of Agronomy.

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tion and was hoed and raked before the beginning of each series of tests. When several runs were made on the same or succeeding days the soil could not be recultivated and was sometimes considerably packed by the applications of water.

The water was applied artificially by means of sprinkling cans to simulate rainfall in the same way that it was applied by Duley and Hays.[5] In three cases the run-off from natural rainfall was collected. In order to get an even distribution of water over the plots several men sprinkled the water simultaneously. With the heavier applications eight men were employed on the 100-foot plot, each man being assigned a 12.5-foot space over which he distributed an allotted quantity of water in a given time. On the shorter plots each man was given 10-foot spaces. By properly calibrating the sprinklers it was possible to put on the water in almost exactly the time desired on all plots.

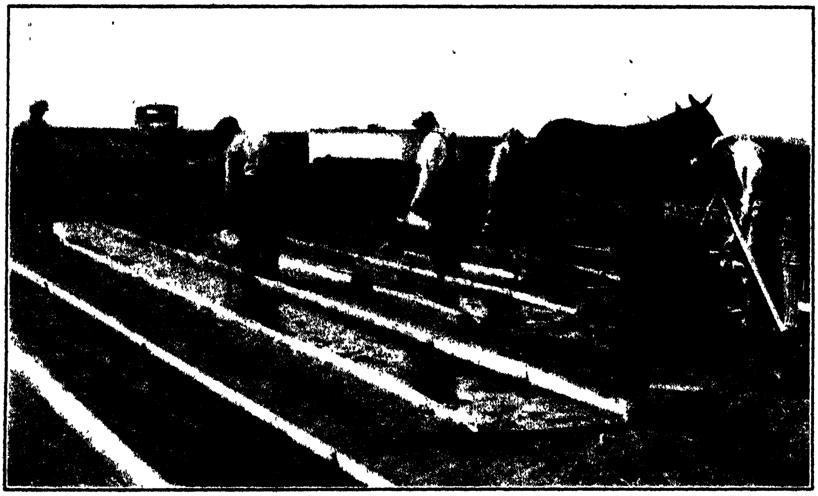

FIGURE 1.—Plots used in experiment 1. A portion of the 100-foot plot is shown at the left, and the 40-, 20-, and 10-foot plots at the right. Three applications of water were made at the rate of 1 inch in 30 minutes.

The losses were determined by weighing the total amount of run-off and then sampling. The samples were oven-dried and the amount of soil and water calculated.

RESULTS

RUN-OFF

A summary of the results obtained will be found in table 1 and figures 2 and 3. These results show that the percentage of run-off was in general greater on the short plots, and decidedly greater when the average of all plots is considered. On two occasions, July 12 and 27 in experiment 2, the 20-foot plot absorbed water much more rapidly than the others. No satisfactory explanation for this has been found, but the greater absorption was very noticeable while the water was being applied. It is felt that these two results should not be included in the data since they were obviously inconsistent at the time. However, averages have been given both with and without them.

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TABLE 1.—*Effect of length of slope on run-off and soil erosion*

EXPERIMENT 1

Date	Water applications or rainfall	Run-off from plots indicated				Soil eroded per acre from plots indicated			
		10-foot	20-foot	40-foot	100-foot	10-foot	20-foot	40-foot	100-foot
		Percent	*Percent*	*Percent*	*Percent*	*Lbs.*	*Lbs*	*Lbs.*	*Lbs*
1931									
Dec 23	1 inch in 30 minutes	0.00	1.26	0.87	0.09		121	21	1
Do	1 inch in 60 minutes	42.69	67.11	55.39	43.47	3,071	5,242	1,757	704
Dec. 26	½ inch in 30 minutes	7.94	6.43	8.56	5.43	160	109	174	86
Do	1 inch in 60 minutes	64.96	74.37	65.72	59.92	378	1,271	1,078	794
1932									
Mar 28	1 inch in 60 minutes	29.80	20.57	15.12	15.36	270	274	134	171
Do	1 inch in 30 minutes	77.03	60.11	58.52	57.04	1,253	2,029	1,073	2,607
Apr 26	0.32 inches rain	25.20	42.70	21.10	16.20	159	558	289	253
May 11	1.26 inches rain	27.60	26.70	19.30	18.30	4,980	3,005	1,230	1,350
Dec 3	1 inch in 30 minutes	40.54	34.90	28.63	25.24	1,086	423	479	299
Do	1 inch in 60 minutes	26.81	24.59	16.75	7.02	244	203	171	60
1933									
Apr 24	1 inch in 30 minutes	1.59	1.43	.81	1.20	18	18	12	30
Do	do	40.38	29.14	26.78	28.15	735	415	491	912
June 6	1 inch in 15 minutes	44.60	31.02	25.56	30.30	271	847	1,085	3,107
June 8	do [a]	37.51	29.73	29.41	25.00	697	900	1,082	2,614
	Average or total	33.33	32.15	26.61	23.77	13,322	15,415	9,076	12,988
	Average for applications of June 6 and 8	41.05	30.38	27.49	27.65	--			---
	Total for applications of June 6 and 8	------	-	-	---	968	1,747	2,167	5,721
	Total for all applications except for those of June 6 and 8	-----		------	-	12,354	13,668	6,909	7,267

EXPERIMENT 2

Date	Water applications or rainfall	Run-off from plots indicated				Soil eroded per acre from plots indicated			
		10-foot	20-foot	40-foot	100-foot	10-foot	20-foot	40-foot	100-foot
1933									
July 8	1.5 inches in 35 [b] minutes (rainfall)	46.42	37.69	30.61	29.22	1,364	3,092	4,403	4,829
July 12 (a m)	1 inch in 60 minutes	21.33	5.98	14.19	7.74	174	102	199	137
July 12 (a m)	1 inch in 30 minutes	55.62	38.01	49.39	36.66	769	718	943	1,000
July 12 (p.m)	do	54.58	34.65	40.45	33.29	943	573	903	1,197
July 19 (a.m)	1 inch in 15 minutes	52.62	40.00	38.38	33.98	4,646	3,920	4,827	4,907
July 19 [c]	do	65.64	57.41	56.79	56.03	5,517	5,009	6,316	5,866
July 27	1 inch in 30 minutes	10.65	7.33	9.30	5.48	116	232	421	160
	Average or total	43.84	31.58	34.20	28.91	13,529	13,646	18,012	18,096
	Average or total omitting results for July 12 and 27 [d]	54.89	45.03	41.93	39.74	11,527	12,021	15,546	15,602

[a] This application followed an application of 1 inch in 30 minutes which gave no run-off.

[b] Only part of 3.25 inches rain collected.

[c] 6 minutes after first application July 19

[d] Omitting results for July 12 and 27 on all plots The 20-foot plot took in water differently from the others on these days and no satisfactory explanation has been found.

When the results are analyzed in detail it will be seen that the run-off from the shorter plots exceeded that from the longer ones in 69 cases out of 84 in experiment 1. If calculations are made according to the method of Salmon,[6] it will be found that D/E = 7.5, which indicates that the difference is probably not due to chance. In experiment 2 the run-off from the shorter plots exceeded that from the longer ones 27 times out of 30, or D/E = 6.6, which again indicates that the difference is not due to chance. If the results from both tests are combined, the run-off from the shorter plots is greater in 96 cases out of 114, or D/E = 10.9, from which it is safe to conclude that a larger percentage run-off may be expected from short slopes than from longer ones, at least up to the lengths used in these tests. This was true during light applications of water such as those of June 6 and 8 and July 19, 1933.

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[6] SALMON, S. C. THE POINT BINOMINAL FORMULA FOR EVALUATING AGRONOMIC EXPERIMENTS. Jour. Amer. Soc. Agron. 22: 77-81. 1930.

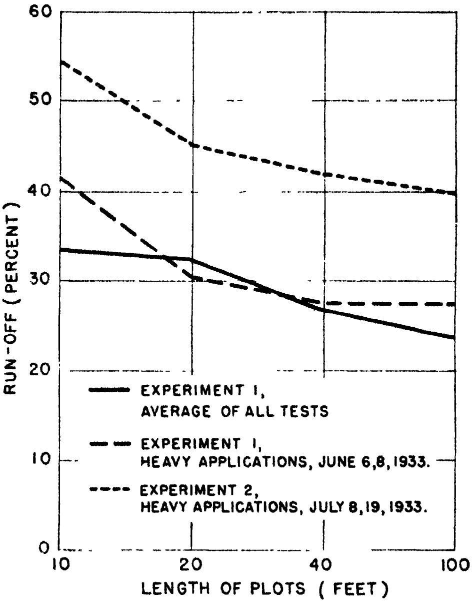

FIGURE 2.—Percentage run-off from soil plots of different lengths.

The explanation for the greater absorption on the longer plots probably lies in the fact that when water is running over a long slope there is more time for it to be absorbed than on a shorter slope. Unless a soil had reached its absolute water-holding capacity this factor would continue to operate. The results of these tests, like those of Duley and Hays,[7] indicate that field soils on sloping ground are seldom if ever so thoroughly saturated that they fail to absorb some water.

The decrease in run-off was less between the 40-foot and 100-foot plots than between 40-foot plots and the 10- or 20-foot plots. This was probably due to the fact that during heavy applications of water much of the lower part of the long plot was covered with a sheet of water. The percentage of run-off was thereby increased since absorption was already going on at approximately the maximum rate. The importance of this factor probably would be greater with still longer plots, and might possibly reach a point where the percentage of run-off would increase with further increase in length of slope, particulary during very heavy rains, for if the entire surface were covered with water, any additional rainfall would give approximately 100 percent run-off.

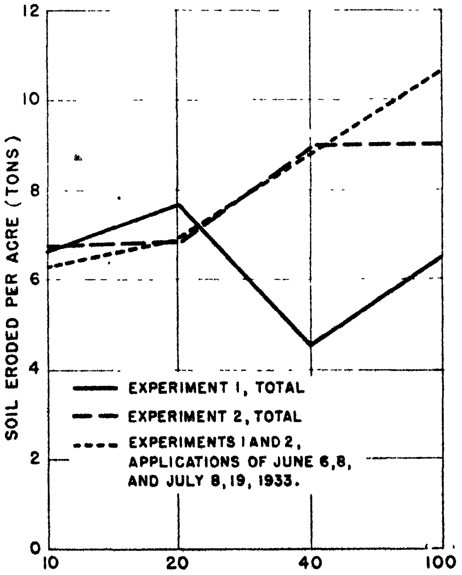

FIGURE 3.—Tons of soil eroded from soil plots of different lengths.

EROSION

The results of the erosion measurements for these plots were less consistent than the results for run-off. If the average of all results in experiment 1 are considered for the different plots it will be seen that the figures for the 10-, 20-, and 100-foot plots are not widely different, but those for the 40-foot plots are low. The large amount of erosion on the 10- and 20-foot plots is due mainly to the results of the test of December 23, 1931, and the rain of May 11, 1932. There seemed to be a general tendency, when the amount of erosion was small, for the rate of erosion per acre to be relatively high for the short plots. This probably can be accounted for by the larger percentage of run-off. When the amount of erosion was large there was a tendency for it to be greater on the longer plots. This probably can be explained by the increased carrying power of water down the longer slope, as the water tends to concentrate in larger volume and to follow definite channels. This increase in volume with its greater cutting power is sufficient to overcome the effect of a larger percentage run-off on the shorter plots. If consideration is given to the times when the applications of water was most rapid, as on June 6 and 8 and July 19, 1933, when water was applied at the rate of 1 inch in 15 minutes, and the rain of July 8, 1933, when 1.5 inches fell in 35 minutes, it will be noted that erosion was heaviest on the longer plots.

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[7] DULEY, F. L., and HAYS, O. E. See footnote 5.

If the results of all tests are considered, erosion from the longer plots is found to exceed that from the shorter ones in 61 out of 114 cases. Since D/E = 1.1, the results do not appear to be statistically significant. However, if the results for the very rapid rains are considered, erosion from the longer plots is found to exceed that from the shorter ones in 27 cases out of 30. D/E = 6.6, which indicates that the results are probably not due to chance. It must, however, be remembered that the numbers used in this case are small, and hence are of less value statistically. Nevertheless they do indicate a definite trend, and since it is the hard dashing rains that cause the greatest amount of erosion, these results have a more direct application to practical conditions.

In general the results here reported indicate that during the lighter applications of water the largest amount of erosion per unit area occurs on the short slopes, but with the heaviest applications the reverse is true. This finding may account for the fact that some of the results obtained in other experiments have shown more erosion from short plots, while in other experiments more was obtained from long plots. The relative amounts seem to depend upon the character of the rainfall.

SUMMARY

Soil erosion and surface run-off were measured on plots 10, 20, 40, and 100 feet in length, to determine the effect of slope length on these processes. Measurements were made on two such sets of plots.

The soil was a silty clay loam, free from vegetation and loose organic matter, and was surface-cultivated. The first set of plots had a slope of 4 percent and the second a slope of 4.4 percent. To simulate rainfall most of the water was applied with sprinkling cans; in a few cases natural rainfall was used.

. There was a larger percentage of surface run-off from the short plots than from the long ones. This seemed to be true with both the heavy and light applications of water for the plot lengths under consideration.

The results for soil erosion were less consistent. When the rate of water application was light, there was a tendency for the erosion from the short plots to run relatively high as compared with the others. When the rate of application was heavy, i.e., 1 inch in 15 minutes, erosion was greater on the long plots. These results indicate that when rainfall is light short plots may possibly undergo the greater erosion, but when rains are heavy the reverse is true.

THE ABSORPTION AND EVAPORATION OF MOISTURE FROM PLANT CONTAINERS[1]

By Linus H. Jones

Assistant research professor of botany, Massachusetts Agricultural Experiment Station

INTRODUCTION

The ordinary flowerpot made of clay and fired is familiar to everybody. Jones[2] has shown that plant containers of metal, glass, and paper will support plant growth equal to, and frequently better than that obtained in clay pots. The real difference between porous and nonporous pots is the property of the former to absorb and evaporate moisture. Soil aeration through the wall of the pot is not probable and is hardly possible.[3]

In seeking a scientific explanation for the inferiority of plants grown in clay flowerpots on a dry surface, the subject of water relations in the soil as influenced by the absorption of water by the pot and its evaporation from the pot seems to give a satisfactory answer. In nonporous plant containers, evaporation can take place only from the surface of the soil. In the porous pots, the pot itself acts as an evaporating surface; and as its area is considerably greater than that of the surface area of the soil, the effects of evaporation from such containers are accentuated. The plant container directly affects the water relationships in the soil mass. Jones, using high greenhouse temperatures, found that the loss of water from 3-inch porous flowerpots was twice as much as from nonporous containers of the same size, and that this increased loss of moisture by evaporation had a cooling effect on the soil mass.

PLAN OF THE INVESTIGATION

The investigation reported herein was planned to secure information that could be used as a scientific basis for the proper understanding of water movement in a clay flowerpot. The experiments were planned to determine the maximum absorbing capacity of clay flowerpots. Painted flowerpots and cement pots were included in the experiments concerned with evaporation. Though porous to air and moisture, the capillary structure of the cement pot is so coarse that unless the soil mass is quite wet, capillary continuity is broken between the cement pot and the soil and the pot will function as a nonporous one.[4]

ABSORPTION OF MOISTURE BY THE CLAY FLOWERPOT

When both the soil and the flowerpot approach dryness, any applied water is taken up by both. Apparently there are no figures to

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[1] Received for publication Nov. 22, 1933, issued June, 1934 Published as Contribution no 178 of the Massachusetts Agricultural Experiment Station

[2] JONES, L. H. FLOWERPOT COMPOSITION AND ITS EFFECT ON PLANT GROWTH. Mass Agr. Expt Sta. Bull. 277, pp. [148]-161, illus. 1931.

[3] ———. AERATION OF SOIL IN PLANT CONTAINERS. Florist Exch and Hort. Trade World 79 (11) 39, illus. 1932.

[4] JONES, L. H. CEMENT FLOWERPOTS . . . Florists' Exch. and Hort. Trade World 80 (2): 9, illus. 1932.

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suggest the approximate amounts of water that should be applied daily to satisfy the needs of potted plants in flowerpots of various sizes. Table 1 shows the maximum amount of absorption that can take place in a clay flowerpot and the relation of the absorbed quantities to the amount of water usually applied to a potted plant.

TABLE 1.—*Normal weight of water applied to clay flowerpots of different sizes, the weight of water that clay pots can absorb, and the weight of water available for the soil and plant*

[Average for 20 pots]

Size of pot (inches)	Normal watering [a] applied	Water absorbed by pot		Water available for soil and plant	
	Grams	*Grams*	*Percent*	*Grams*	*Percent*
2	15	9.10	60	5.90	40
2½	35	14.10	40	20.90	60
3	50	20.55	41	29.45	59
4	100	38.85	39	61.15	61
5	200	83.15	42	116.85	58
6	300	130.30	43	169.70	57

[a] The amount of water that constitutes a normal watering was determined by averaging data from several sources The amount of soil in the pot and the human element in watering will cause these figures to vary slightly. However, they represent a fair basis for investigational work.

A cement pot made from a mixture of sand and cement does not have so large a water-holding capacity as a flowerpot made of fired clay, consequently a larger percentage of the water applied to the soil in a cement pot is available for the plant. Table 2 shows a comparison of the absorbing capacities of clay and cement flowerpots.

TABLE 2.—*Normal weight of water applied to clay and cement flowerpots of different sizes, the weight of water absorbed, and the weight available for the soil and plant*

[Average for 2 pots]

Size of pot (inches)	Type of pot	Dry weight of pot	Normal watering applied	Water absorbed by pot		Water available for soil and plant	
		Grams	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*
4	Clay	390	100	37	37	63	63
	Cement	406	100	23	23	77	77
5	Clay	643	200	83	41	117	59
	Cement	618	200	44	22	156	78
6	Clay	1,060	300	121	40	179	60
	Cement	1,032	300	79	26	221	74

In order to determine the amount of water that could be absorbed by a clay pot in the presence of a growing plant, the following experiments were performed: Twenty-four 4-inch pots were dried for 1 day at an air temperature of 33° C. and weighed. Each pot was planted with a geranium plant and placed in a greenhouse on a moist soil bench. At the end of 6 weeks the potted plants were transferred to a dry surface and the watering adjusted so that the soil was almost dry as the plants began to wilt. (This procedure is analogous to the conditions under which house plants are grown and watered.) When all 24 plants were reacting nearly the same and needed watering, they were equally divided into two series. The plants and soil were removed from the A series and the pots weighed to determine how much water was retained by the pots. From table 3 it appears that the pots were as dry as when the experiment was started. To each pot of the B series 100 grams of water was applied and the series allowed to stand for 1½ hours. The pots with soil and plants were weighed and the loss of moisture by transpiration and evaporation determined. The soil and plants were then removed and the wiped pots weighed. Table 3 gives averages and calculations on which are based the conclusion that nearly one quarter of the water applied was absorbed by the pots. This figure represents about one half of the absorbing capacity of the pots (table 1). It was observed that in 1½ hours the water had penetrated the soil to a depth of but 1 inch and probably would not have penetrated more deeply as the capillary attraction of the pot for moisture was greater than that of the soil.

TABLE 3.—*Method of grouping the various weights of clay pots in order to obtain a general average of the percentage of water absorbed by them when containing plants*

Series and no.	Item considered	Weight
Series A (check):		*Grams*
1	Average initial weight of 12 pots	394.00
2	Average final weight of pots minus soil and wilted plants	393.00
Series B.		
1	Average initial weight of 12 pots	390.75
2	Average weight of pots plus soil when soil was dry and plants wilting	812.08
3	Average weight plus 100 grams of water	912.08
4	Average weight 1½ hours after water was applied	905.91
5	Loss in 1½ hours due to evaporation and transpiration	6.17
6	Average weight of empty pots 1½ hours after water was applied	413.33
7	Average weight of water in pots after 1½ hours (413.33 − 390.75)	[a] 22.58

[a] 22.58 percent of the total weight of water applied was absorbed by the clay pots in 1½ hours.

EVAPORATION OF MOISTURE FROM POROUS AND PAINTED FLOWERPOTS

Moisture loss from the soil in a porous pot takes place in two directions—the evaporation from the surface of the soil causes a vertical movement of soil moisture while the evaporation from the wall of the pot causes a lateral movement. If the porous pot is kept on a moist bench surface, some of this evaporated moisture is replaced from the moisture below the pot. The following experiment was conducted to determine the effect of pot environment (bench surface and air) on the quantity of water lost by evaporation from the surface of the soil and through the wall of the pot.

Flowerpots, 5-inch size, of clay, cement, and painted clay were placed on a dry bench surface of boards and on a moist bench surface of soft-coal cinders. Each type of pot for each condition was replicated six times. The soil, a compost, was sieved through a 1¼-inch mesh screen. The pots were filled level full and the soil firmed. The drainage hole in each pot was covered with a piece of broken pot, as is customary. The pots were allowed to remain on a moist surface for 20 hours for the natural adjustment of moisture through the soil before being weighed and were brought up to this initial weight every 24 hours, after first determining the weight lost. Table 4 shows the loss of weight for each one of the 14 days of the experimental period. Naturally, the amount of evaporation is affected by the weather conditions prevailing at the time. This influence is graphically represented in figure 1.

TABLE 4.—*Average loss of weight in 24-hour periods from clay, painted, and cement soil-filled pots on dry and moist surfaces*

Period (days)	Clay pots		Painted pots		Cement pots	
	Dry surface	Moist surface	Dry surface	Moist surface	Dry surface	Moist surface
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	53	18	13	11	27	5
2	63	29	17	16	30	21
3	64	28	15	11	27	18
4	66	36	19	18	31	25
5	69	41	18	18	33	23
6	53	26	15	11	27	18
7	54	18	16	11	30	[a] 2
8	56	23	18	14	29	14
9	28	5	10	8	19	[a] 10
10	53	17	17	12	27	5
11	44	15	14	11	24	7
12	48	14	14	12	22	5
13	11	[a] 6	4	4	5	[a] 10
14	20	5	7	5	11	[a] 1
Total	4,094	1,630	1,184	947	2,064	646

[a] Gain in weight.

The results indicate that the evaporation of moisture from porous clay pots on a dry surface was approximately three times greater

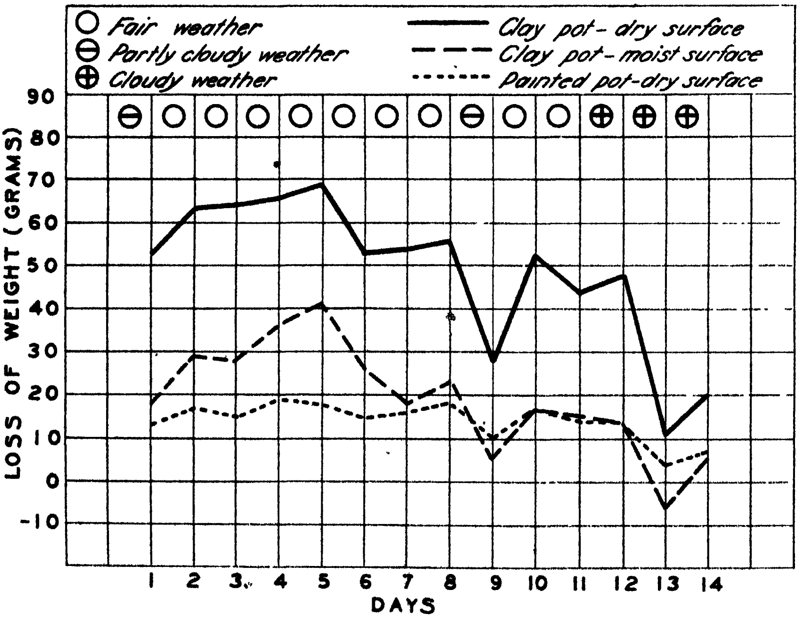

FIGURE 1.- Effect of weather on loss of weight of clay pots on dry and moist surfaces and painted pots on a dry surface. Average for six pots.

than that from painted pots. As the painted pots could evaporate moisture only from the soil surface, the data show that two thirds of the loss of moisture from the soil in a clay pot on a dry surface took place through the wall of the pot, indicating that there was twice as much movement of moisture laterally as vertically. When the porous clay pot was kept on a moist surface, a considerable proportion of the water lost by evaporation was replaced from the moist surface. It is also possible in humid weather for the porous clay pot to absorb more moisture from the moist bench than is lost by evaporation.

The cement pot, because of its coarse structure, influences loss of moisture according to the ability of soil moisture to leave the soil and enter the pot wall. When moisture was first applied at the beginning of a 24-hour period, the pot was able to absorb it. As the period progressed, the moisture-retaining power of the soil became more effective than the pull of the pot. Thus capillary contact between soil and pot was broken. Hence, soil-moisture movement in a cement pot is more like that in a painted pot, vertical, than in a porous clay pot, vertical and lateral. Table 5 shows the loss of moisture in 1-hour periods from the three types of pots. The transition period, when the cement pot (on a dry surface) changed over from behaving like a porous pot, occurred at about the end of the fifth hour.

TABLE 5.—*Loss of weight in hourly periods from clay, cement, and painted soil-filled pots on dry and moist surfaces*

Period (hours)	Dry surface			Moist Surface		
	Clay	Cement	Painted	Clay	Cement	Painted
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
0-1	5	1	4	1	0	1
1-2	8	8	2	4	5	1
2-3	2	4	3	5	4	2
3-4	12	7	2	3	3	3
4-5	10	6	2	6	5	2
5-6	10	2	2	7	1	3
6-7	1	5	4	2	5	3
7-8	5	1	0	2	3	0
8-24	45	12	10	22	14	13
Total loss	98	46	29	52	40	28

DISCUSSION

The absorption of moisture by the clay pot and its subsequent evaporation into the air is a continuous process as long as there is any capillary moisture in the soil mass. Tests with the rubber vacuum disk proved that the word "porous" as applied to moist clay flowerpots should be limited to mean porosity to moisture. Air does not pass though the wall of a moist clay flowerpot. The process of lateral movement of moisture transports more water in this direction than is moved vertically and should, therefore, be accompanied by a corresponding lateral movement of soluble plant food. Associated with the movement of water in plant containers is the distribution of the root system of the plant. Where lateral movement is possible through a porous pot, the root system, for the most part, is found between the wall of the pot and the soil mass. On the other hand, if lateral movement is not possible, the root system ramifies through the soil mass with only a few roots developing adjacent to the pot wall.

Water relations, water movement, and root distribution are entirely dependent on the structure of the plant container. The culture of potted plants should include practices that encourage a more or less even balance of water content in the environment of the roots. In a nonporous pot with a ramifying root system and lack of lateral movement of water, it is comparatively easy to maintain an even distribution of moisture in the soil mass. In a porous pot where the root system is almost entirely against the wall of the pot, it seems almost necessary to keep such pots on a moist surface. If a moist surface is not used, the pot itself will withdraw moisture from the nearest source which happens to be the very region where the roots have developed or are developing.

SUMMARY AND CONCLUSIONS

This investigation is concerned with the amounts of water usually applied to potted plants and the moisture-holding capacities of pots of various sizes. The loss of water by evaporation has been determined also for pots in certain specified environments.

Under conditions that frequently prevail with growing plants, nearly one quarter of the amount of water applied was absorbed by the flowerpots in 1½ hours.

Twice as much water was evaporated from the wall of a clay pot as from the surface of the soil. This would indicate that there was twice as much moisture moved laterally as was moved vertically.

When the clay pot was kept on a moist surface, a considerable proportion of the evaporated water was replaced from the supply of moisture beneath the pot.

Cement flowerpots did not have so large a water-holding capacity as those made of fired clay. The cement pot evaporated moisture from the wall as long as the wall could maintain a capillary connection with the soil mass. When such capillary contact was broken, the cement pot behaved as a nonporous painted pot.

The essential difference between a porous and a nonporous plant container is the ability of the former to evaporate moisture from its wall and replace this moisture from the soil moisture within the pot.

THE RELATION BETWEEN ABNORMAL ORIENTATION OF THE 4-DAY EMBRYO AND POSITION OF THE CHICK AT HATCHING[1]

By J. R. CAVERS, *research assistant in poultry husbandry*, and F. B. HUTT, *poultry husbandman and animal geneticist, Minnesota Agricultural Experiment Station*

INTRODUCTION

Despite the extensive knowledge at hand concerning the early embryology of the chick and the steadily accumulating information regarding its behavior at the time of hatching, there is very little known of the relationships which exist between the two stages of development. Because of their importance as factors causing embryonic mortality, malpositions of the fully formed embryo have been the object of study at this station for several years. The data herein presented deal with abnormal orientations of the young embryo and their possible effects upon the subsequent position of the chick within the egg.

MALPOSITIONS

The completion of the incubation period usually finds the body of the embryo parallel to the long axis of the egg, with the head towards the large end of the shell. It has been generally considered that the embryo accommodates itself thus to the shape of the egg at the end of the second week, but Kuo (*9*),[2] who has described the manner in which the final position is attained, found that the time of its fixation is usually about the tenth day.

Several well-defined deviations from the normal position at hatching, referred to as malpositions, have been described.

The method of designating normal and abnormal positions of fully formed embryos at this laboratory is as follows:

N. The normal position. Body parallel to long axis of egg and head in large end; beak under right wing and toward air cell (fig. 3, *A*).
I. Head buried between thighs.
II. Embryo upside down with head in small end of egg (fig. 3, *B*).
III. Head turned to left and away from air cell.
IV. Body rotated so that head is away from air cell; otherwise normal.
VI. Head not beneath wing, but above or away from it.

More complete descriptions of these malpositions, data on their frequencies, and discussions concerning their relation to embryonic mortality, have been given by Sanctuary (*12*), Hutt (*6*), and others. A detailed description of position VI has been given by Hutt and Pilkey (*7*). The malpositions differ in the extent to which they prevent or hinder hatching. Position I is undoubtedly always lethal, and positions III and IV appear to be fatal in nearly all cases, if not in all. Some of the embryos in positions II are able to hatch. Position VI is probably a considerable hindrance to the chick, if not an actual barrier to hatching.

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[1] Received for publication Nov. 8, 1933; issued June, 1934. Paper No 1224 of the Journal Series of the Minnesota Agricultural Experiment Station. The sixth paper of the series of "Studies in embryonic mortality in the fowl."

[2] Reference is made by number (italic) to Literature Cited, p. 530.

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Although some chicks in malpositions may hatch and their positions then be unknown, the influence of abnormal orientations can still be studied in the embryos which fail to hatch.

In the first paper of this series, Hutt (*6*) suggested that some of the abnormal positions of fully formed embryos may result from the aberrant orientations of the embryo, which several embryologists have shown to be not infrequent, and which are known to be established at early cleavage. Such an origin seemed especially likely in the case of position II (head in small end of egg) since very young embryos are sometimes found parallel to the long axis of the egg (instead of at right angles to it), with the head pointing toward the small end. Accordingly an investigation was begun in 1931 to determine whether or not any relation exists between the early orientation and the later position of the embryo. Since the investigation was begun Taylor (*13*) has reported that from embryos originally abnormally oriented there resulted 50 percent more embryos in position II than there were from embryos having normal orientation at 6 days.

ORIENTATION

The position of the avian embryo on the yolk during early stages of development has a fairly definite relation to the axes of the egg. When an egg remains in a horizontal position for a few moments, the yolk comes to rest with the blastoderm on top. The embryo may then readily be observed by removing a portion of the shell above it. Embryos of the domestic fowl at 2 or 3 days of age were described by Von Baer (*1*) as lying at right angles to the long axis of the egg, with the head to the left when the small end of the egg is directed away from the observer. Early embryologists considered the normal orientation given by Von Baer to be relatively constant in all eggs, and used it in studies of segmentation as a means of predicting the future caudal and cephalic regions of the undifferentiated blastoderm. Later investigations have shown that marked deviations may occur from the normal orientation. Féré (*4*) measured orientations of a large number of chick embryos and found that 25 percent of them deviated by more than 45° to the right or left of the normal axis, This is in agreement with the findings of Dalton (*3*), Rabaud (*10*). Kopsch (*8*), and Taylor (*13*).

PROCEDURE

Since it was necessary not only to determine the orientation but also to give the embryo an opportunity to hatch, it was essential that the position of the embryo at an early age be determined without breaking the shell as had been done in most previous studies. Moreover, it was desired to obtain the readings of orientation as early as possible in the incubation process in order to ascertain more nearly the original orientation established during cleavage. For these reasons special apparatus had to be designed to permit observation of young embryos through the shell.

APPARATUS

A candling apparatus was constructed which contained two 500-watt projection lamps and an electric fan to cool them. The box was designed to give the maximum illumination of an egg placed in a horizontal position in an egg-shaped hole in the top. Very little light could emerge except through the egg. The eggs of White Leghorn fowls were used, since their chalk-white shells transmit the light readily.

The top of the box was marked with lines which radiated out from the center of the egg-shaped hole every 15° throughout an entire circle. The line which represented the normal orientation of the embryo, being perpendicular to the long axis of the egg and 90° counterclockwise from the small end, was marked 0°. Other lines were marked 15°, 30°, 45°, etc., to the right or left of this line (fig. 2).

DETERMINATION OF ORIENTATION

The apparatus just described made readings of orientations possible at 3 days of incubation. The vitelline blood vessels of the normal embryo showed fairly well through the shell at 60 hours, and soon thereafter the body itself was visible. At 72 hours the cephalic and caudal regions could be distinguished, and in many cases the beats of the heart as well. Since the cranial and cervical flexures are at that age well advanced, the embryo itself is not entirely suitable for accurate measurements. It was found that the vitelline veins and arteries could be utilized along with the body of the embryo in determining the angle of orientation.

After about 72 hours of incubation the vitelline blood vessels were distinctly visible through the shell. These pass bilaterally from the embryo at right angles to its long axis and divide into two main branches, which extend in anterior and posterior directions. The forward branches develop more readily to form a wide fork, the arms of which are more or less parallel to the body of the embryo. The fork formed by the posterior branches occupies a similar relation to the embryo, but it is not so clearly defined until about 80 hours. By 90 hours, however, these vessels may have attained an equal or even greater length than those extending forward (fig. 1).

Since the object of the experiment was to determine the relationship between the definitive position just prior to hatching and the original orientation established in the early cleavage stages, it was desirable to determine that orientation at the earliest possible age consistent with accurate readings. After numerous trials this was found to be between 84 and 90 hours. At that time the embryo and the blood vessels both serve to differentiate the two ends of the body. The angle of orientation is indicated more exactly by the blood vessels than by the embryo itself, for the reason that they extend farther over the yolk and they are more plainly visible through the shell. The extra-embryonic blood vessels are less subject to changes of position than is the embryo, especially when the latter is enclosed within the amnion from the fourth day on. Moreover, it was found by breaking eggs at 48 hours that the branches of the blood vessels described above are almost exactly parallel to the straight line which the embryo's body then forms.

In eggs incubated in a horizontal position, the 4-day embryo is found on top of the yolk and near the highest point of the shell. Readings were quickly made in such eggs directly upon removal from the incubator. A number of the eggs used were incubated with the large end up, and the long axis of the egg about 45° from the vertical. In these some difficulty was at first encountered because, as the eggs cooled, the embryonic membranes adhered to the inner shell membrane in the region of the air cell. This obstacle was easily overcome by placing all eggs in a horizontal position immediately upon their removal from the incubator, a procedure which caused the yolk to float freely so that the orientation of the embryo could be determined without difficulty. Readings were made to the nearest 15° radius.

DISTRIBUTION OF ORIENTATIONS

The orientations of 4,721 chick embryos were observed by candling on the fourth day of incubation. Of these 2,906 were in eggs incubated in a horizontal position and 1,815 were in eggs incubated in the tilted position described above. Figure 2 shows the distribution of all these embryos at 24 different angles on the yolk.

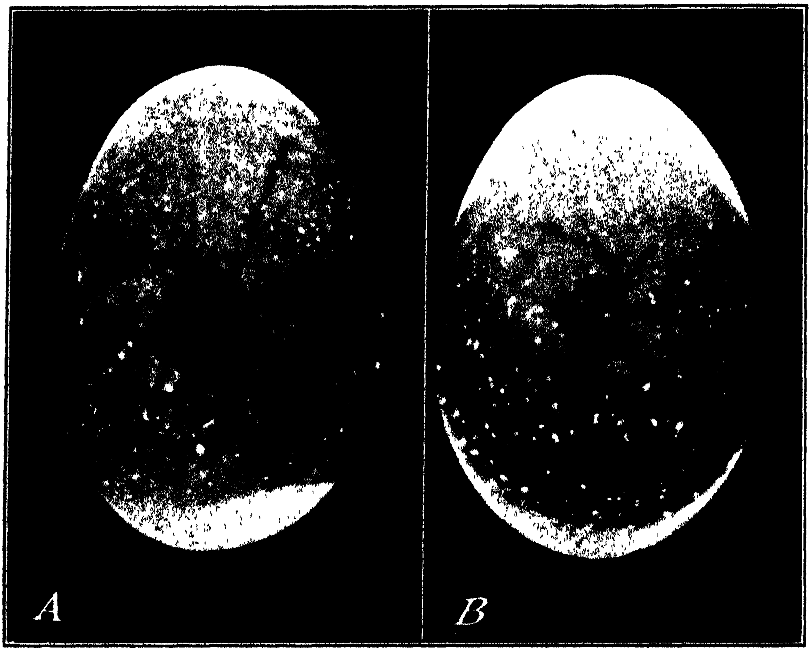

FIGURE 1. - Photographs of living chick embryos at 90 hours of incubation, taken through the shell of the egg. Owing to slight movements during the 10-minute exposures used in taking these pictures, the bodies of the embryos are less distinct here than when orientations were measured: *A*, Normal orientation; *B*, an embryo deviating to the right of the normal orientation. In both cases the anterior end of the embryo is to the observer's left.

The skewed distribution shown in figure 2 indicates that deviations occur more often to the right of the normal axis, i.e., toward the small end of the egg, than to the left. A similar condition is apparent also in the rather limited numbers reported by Dalton (*3*) and Kopsch (*8*). Unfortunately Féré's extensive data are not presented in sufficient detail to permit a comparison. Other data in this laboratory indicate that this skewed distribution of orientations arises from differences between individual hens with respect to the early orientations of their progeny.

The class with the center at 0° and a range of 7.5° on each side contains 1,491 embryos, or about one third of the total number.

Obviously the definition of normal orientation cannot be limited to such a narrow class. It has seemed preferable to consider as normal all orientations falling within the quadrant bounded by radii at 45° right and left of the so-called normal axis at 0° (fig. 2). The classes exactly at 45° right and 45° left contain 480 and 117 embryos, respectively. These cannot all be classed either as normal or abnormal, but by allotting them to the groups of normal and abnormal orientations in the proportion of 566:291 (right) and 243:76 (left)—the frequencies in the two adjacent classes of orientation—it was possible to determine the number belonging in the normal quadrant.

This permitted a comparison of the writers' determinations with those of Féré (*4*), Rabaud (*10*), and Kopsch (*8*), which is shown in table 1. It is evident from these data that with large numbers of chick embryos 75 percent of their orientations are to be expected within the normal quadrant.

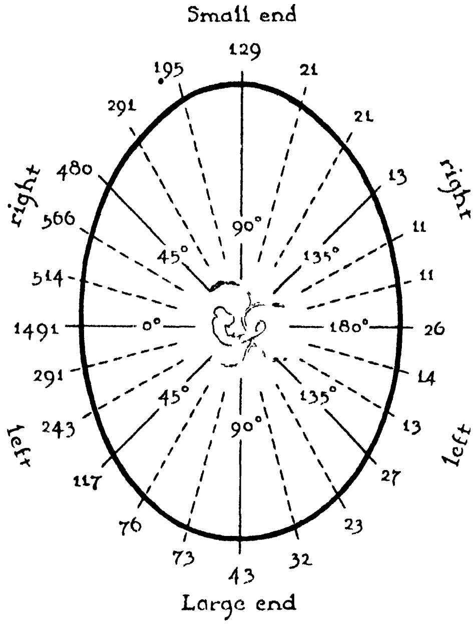

FIGURE 2.—Distribution of 4,721 chick embryos oriented at different angles with respect to the principal axes of the egg at 4 days of incubation

TABLE 1.—*The proportion of normal orientations in the present data compared with those in other investigations*

Investigators	Method of observation	Total orientations	Orientations within normal quadrant
		Number	*Percent*
Cavers and Hutt	Through shell	4,721	74.4
Féré (*4*)	Shell removed	2,926	74.9
Kopsch (*8*)	do	155	62.6
Rabaud (*10*)	do	100	76.0

The accuracy of the method was tested by making a second measurement on 204 embryos 12 hours after the first. The eggs were turned in the incubator between readings, a procedure which would change the orientation if that were not relatively constant. The results, shown in table 2, indicate that the method used is remarkably accurate. Moreover, it has been shown (table 1) that the type of distribution is similar to that obtained by other workers. It is concluded therefore that the method of observing positions through the shell and using largely the vitelline blood vessels to indicate the angles of orientation gives essentially the same result as when the body is observed directly after breaking the egg. It has the special advantage of not interfering with the viability of the embryo.

TABLE 2.—*Comparison of two readings of orientation taken 12 hours apart on the same embryos, the eggs having been turned between readings*

Orientation	Eggs in indicated deviation class	
	Number	*Percent*
Same orientation at both readings	109	53.4
Deviation of 15°	80	39.2
Deviation of 30°	13	6.4
Deviation of 45°	2	1.0
Total	204	100.0

ORIENTATION AND SUBSEQUENT MORTALITY

In order that the dead embryos might be examined in good condition and the age at death estimated, the eggs were candled several times throughout the hatch. Of the 4,721 embryos whose orientations were measured on the fourth day, 3,864 were utilized in the study of subsequent mortality. The distribution of mortality among these during three subsequent periods of incubation and the total mortality are presented in table 3. The percentages of mortality in any one period e.g., 11 to 17 days, are based on the number of live embryos in each of the different classes of orientation at the beginning of that period. The percentages of total mortality are calculated on the original 3,864 embryos alive on the fourth day.

TABLE 3.—*Embryonic mortality at different periods of incubation in embryos originally having different orientations*

Orientation on fourth day (degrees)	Mortality in each period based on the number of embryos alive at the beginning of that period				Total mortality, 4-21 days	
	Embryos	4-10 days	11-17 days	18-21 days		
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
135±22.5, left	54	5.6	0	5.9	6	11.1
90±22.5, left	131	4.6	4.8	20.1	36	27.5
45±22.5, left	357	3.9	4.7	17.1	86	24.2
0±22.5	1,971	5.0	5.0	15.5	467	23.7
45±22.5, right	1,002	7.5	5.7	17.3	279	28.0
90±22.5, right	271	5.5	5.9	20.3	79	29.2
135±22.5, right	38	7.9	2.9	14.7	9	23.7
180±22.5	40	2.5	7.7	16.7	10	25.0
Total or average	3,864	5.6	5.1	16.4	972	25.2

Of 3,461 embryos which survived to the nineteenth day, 222 were then removed and examined without being allowed to hatch. The expected 18-to-21-day mortality for these has been calculated to the nearest whole number in each class of orientation at the rate prevailing for that class in the same period among the remaining 3,239 embryos. For example, 26 of those removed had originally been oriented at 45° ± 22.5° left. The mortality after 18 days among 301 embryos originally so oriented, but given an opportunity to hatch, was 52, or 17.3 percent. Accordingly, the expected mortality for the 26 removed was 4 and the total 18-to-21-day mortality in the class was taken as 56. The number among which mortality was actually observed was so large that the calculation for the comparatively small number removed is quite justifiable.

For convenience, and to avoid fluctuations arising from small numbers, the embryos have been grouped in 8 classes of orientation. Each of these 8 includes 3 of the original 24 classes used when readings were made. For example, the class at 45° ± 22.5° right includes those embryos originally at 30°, 45°, or 60° to the right of the normal position, and, since these are mid-class values, the actual limits of the large class are at 22.5° right and left of the radius at 45° right.

The distribution of mortality is fairly uniform throughout the different classes of orientation except where the numbers are small to begin with. Embryos in the class at 135° ± 22.5° left are especially favored, the rate of total deaths being less than half that found in the rest of the population. This might indicate an advantage to the embryo in being oriented toward the air cell of that egg were it not for the fact that in the class at 90° ± 22.5° left, mortality is slightly in excess of that of the total population. High or low death rates in any of the classes with few embryos are accompanied by compensating fluctuations in adjacent classes and may therefore be attributed to chance. It is worthy of note, however, that the lowest total mortality (except in two classes containing small numbers) occurs in the modal class (0° ± 22.5°), and that this class is the one containing embryos deviating not more than 22.5° from the orientation considered the norm for this species. A comparison of the mortality rate in this group with that for all other embryos reveals the following figures:

	Mortality (percent)
Normally oriented (1,971)	23.69 ± 0.97
All others (1,893)	26.67 ± 1.02
Difference	2.98 ± 1.40
Difference / Standard error	2.1

Since the difference between the mortality rates for these two groups is 2.1 times its standard error, it is statisticially significant, and since the rates for every class of abnormal orientation having adequate numbers are uniformly higher than that for the normal class, it may also safely be considered as biologically significant. This agrees with the finding of Taylor (*13*) that eggs oriented normally at 6 days hatched better than others by about 5 percent. The normal orientation is evidently slightly more conducive to the survival of the embryo than are deviations from it.

It is obvious that the excessive mortality among the abnormally oriented embryos occurs in the last 4 days of incubation and that it is to a large extent attributable to the comparatively high rate of 29.2 percent prevailing for embryos originally oriented at right angles to the normal orientation and with the head toward the small end of the egg (90° ± 22.5° right). The number in this class, however, is too small to indicate by statistical methods that its mortality rate is significantly higher than that for the normal class (23.7 percent) or for the entire population (25.2 percent). The excessive mortality in this class occurred chiefly in the last 4 days of incubation, and it will be shown later that it resulted from an excess of the head-in-small-end malposition.

The same class of orientation also exhibited high mortality during the period from 11 to 17 days. More extensive data would be necessary to prove that these embryos are under a handicap during this period, but such a possibility is indicated. Apart from this the mortality prior to the eighteenth day of incubation does not appear to be related to the original orientation of the embryo.

ORIENTATION AND MALPOSITIONS

All eggs failing to hatch were examined on the twenty-second day. The positions were recorded of embryos which appeared to have survived beyond the eighteenth day of incubation, as indicated by size and by the amount of yolk enclosed within the body. Of 3,239 embryos which survived to the eighteenth day or beyond and which were given the opportunity to hatch, 533 failed to emerge from the shell. Some were alive and even pipped, but since they had not hatched on the twenty-second day they were considered as dead. Almost 65 percent of the 533 embryos were in one or other of the five malpositions described on page 517.

The early orientation of each embryo which died at 18 to 21 days is presented in table 4. Included with the frequency of each position at that age (o) is the expected frequency (c). The latter numbers were derived by allocating the embryos dying in a given position at 18 to 21 days to the various classes of orientation according to the proportions of the 3,239 embryos in those same classes at 4 days. If abnormal orientation has no relation to the occurrence of malpositions, there should be no significant difference between the observed and calculated distributions. The results of tests for goodness of fit are given in the two lower lines of the table. In making these calculations the classes having small numbers were combined, as is recommended by Fisher (*5*), to avoid the use of an expected number lower than 5. The values of *P* obtained for the distributions of positions normal, I, III, IV, and VI are well above the 0.05 level of significance, and differences between the observed and expected distributions of these positions may therefore be ascribed to chance. On the contrary, the distribution of position II gives a *P* value much smaller than 0.01, and the difference between the observed and expected values in this case is therefore highly significant. This means that more embryos in position II are associated with certain orientations than would be expected from chance alone.

TABLE 4.—*The early orientations of 533 fully formed embryos found dead in normal and abnormal positions at age of 18 to 21 days*

Orientation on fourth day (degrees)	Observed (o) and expected (c) frequencies of positions											
	Normal position		Position I		Position II		Position III		Position IV		Position VI	
	o	c	o	c	o	c	o	c	o	c	o	c
135±22.5, left	----	2.7	----	0.9	1	1.0	2	0.8	----	0.5	----	1.7
90±22.5, left	5	6.8	7	2.4	2	2.4	1	1.9	----	1.2	8	4.3
45±22.5, left	15	17.7	7	6.1	5	6.3	5	5.0	5	3.2	15	11.2
0±22.5	101	98.0	29	34.0	19	35.1	29	27.8	18	17.5	62	62.4
45±22.5, right	50	47.6	15	16.5	26	17.0	11	13.5	10	8.5	28	30.3
90±22.5, right	15	13.3	8	4.6	13	4.8	4	3.8	1	2.4	5	8.5
135±22.5, right	2	1.9	----	.7	1	.7	2	.6	----	.3	----	1.2
180±22.5	2	2.0	----	.7	1	.7	----	.6	----	.4	3	1.3
Total	190	190.0	66	65.9	68	68.0	54	54.0	34	34.0	121	120.9
χ^2	2.34		5.72		19.76		0.73		0.77		3.64	
P	.80		.22		$<.01$		.68		.68		.46	

The relation of abnormal orientation to the occurrence of position II is clearly demonstrated in table 4. The observed occurrences of this malposition are deficient in the 0° class and excessive in classes at 45°

and 90° right. It will be recalled that 90° right represents an orientation toward the small end of the egg and that similarly in malposition II the chick is upside down with its head in the small end of the egg (fig. 3, *B*). Abnormal orientation at an early stage is not the sole cause of position II, however, for 19 of the 68 embryos dying in that position at 18 to 21 days had been normally oriented on the fourth day, and 8 had actually been directed toward the large end of the egg.

The relation of abnormal orientations to subsequent positions of the embryo at hatching may also be measured in another way by considering what eventually happened to those originally having different orientations. The only limitation in such an analysis is that the positions of hatched chicks cannot be known with certainty and the analysis must be confined to those found dead at 18 to 21 days of incubation. Only the results for position II are presented (table 5) because early orientation apparently had no relation whatever to the occurrence of the other malpositions. In table 5 the frequency of embryos dead in position II for each class of orientation is expressed as a percentage of the total number of embryos which were in that class on the fourth day and which survived to 18 days.

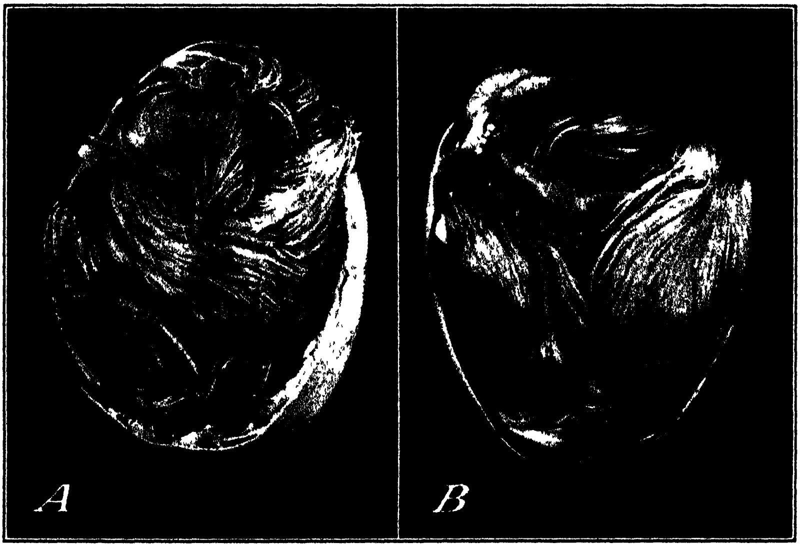

FIGURE 3.—Fully formed chicks which failed to hatch *A*, Normal position, the head under the right wing and the beak just entering the air cell; *B*, position II, the embryo completely reversed with the head in the small end of the egg.

TABLE 5.—*Frequencies of position II in unhatched eggs, expressed as percentages of the 18-day live embryos in each class of original orientation*

Orientation on fourth day (degrees)	18-day embryos	Embryos dead in position II	
		Number	*Percent*
	Number		
135±22.5, left	46	1	2.2
90±22.5, left	116	2	1.7
45±22.5, left	301	5	1.7
0±22.5	1,670	19	1.1
45±22.5, right	811	26	3.2
90±22.5, right	227	13	5.7
135±22.5, right	33	1	3.0
180±22.5	35	1	2.9
Total	3,239	68	2.1

The 68 cases of position II found among chicks failing to hatch represent 2.1 percent of all the embryos alive at 18 days. While the frequency of this malposition among the 1,670 embryos alive at 18 days and originally normally oriented (0° ± 22.5°) was only 1.1 percent, its incidence among 1,569 similar embryos originally oriented otherwise was 3.1 percent, and among those originally oriented with the head toward the small end (90° ± 22.5° right) it attained the maximum frequency of 5.7 percent. This means that, apart from all other causes of mortality, the chance of dying in the head-in-small-end malposition during the last 4 days of incubation was, in the writers' material as a whole, over five times as great for the embryo directed toward the small end of the egg at 4 days of incubation as for the one not deviating more than 22.5° from the normal orientation at the same age. It will be shown later that this chance was lower among eggs incubated in one position than for those in another.

Taylor (*13*) has reported finding 50 percent more embryos in the head-in-small-end malposition at hatching time among those abnormally oriented at 6 days than among those normally oriented at the same age. From his abstract it would appear that all those not deviating more than 45° were considered as normal, and on this basis his results would seem comparable to those just given.

Further and somewhat more direct evidence of the influence of abnormal orientation upon the occurrence of malposition II is furnished by the data for 222 embryos which were all examined on the nineteenth day, and none of which were allowed to hatch (table 6). Position II occurred in 6.3 percent of these embryos. The numbers involved are small for the purpose of calculating percentages, but they do illustrate the marked tendency of embryos oriented toward the small end of the egg on the fourth day to be in a similar position at hatching.

TABLE 6.—*Occurrence of position II in embryos of known orientation, from eggs broken and examined on the nineteenth day*

Orientation on fourth day (degrees)	19-day embryos	Embryos in position II		Orientation on fourth day (degrees)	19-day embryos	Embryos in position II	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
135±22.5, left	5	1	20.0	90±22.5, right	14	5	35.7
90±22.5, left	3	0	0	135±22.5, right	1	0	0
45±22.5, left	26	0	0	180±22.5	1	0	0
0±22.5	109	4	3.7				
45±22.5, right	63	4	6.3	Total	222	14	6.3

The higher frequency of position II among these embryos than in those failing to hatch might be taken to indicate that a considerable proportion of chicks in this malposition are able to hatch were it not that the numbers involved are too small to permit a conclusion and that these eggs were all incubated in the horizontal position. As will be shown later, this position during incubation is particularly conducive to the assumption of the head-in-small-end malposition.

On the other hand, 9 of the 14 embryos originally oriented directly toward the small end of the egg (class 90° right, table 6), had succeeded in correcting their position before the nineteenth day. Of the

78 embryos oriented in the half of the egg away from the air cell (45° 90°, and 135° right) only 9, or 11.5 percent, retained that position. It is evident, therefore, that only a small proportion of these abnormal orientations have resulted in position II. Other malpositions in these same 222 eggs opened on the nineteenth day occurred independently of the early orientation.

REDUCTION OF THE RISK FOR ABNORMALLY ORIENTED EMBRYOS

In the fifth paper of this series Hutt and Pilkey (7) showed that position II is significantly more frequent in eggs incubated in a horizontal position than in those tilted at 45° with the large end up. The question naturally arises whether the tilted position makes it easier for all embryos to assume the correct position for hatching or whether it gains its advantage chiefly by facilitating the escape from the head-in-small-end malposition of the embryos originally oriented toward the small end of the egg. Analysis of the material upon which the present paper is based sheds some light upon this problem.

Of the 3,864 embryos of known orientation which were utilized in the study of subsequent mortality and malpositions, 2,049 were incubated in the horizontal position and the remainder in the tilted position, all being horizontal after the eighteenth day. In table 7 there is shown the effect of the tilted position upon the incidence of position II in embryos originally oriented (1) at 90° ± 37.5° right and (2) any and all other ways including the normal orientaton at 0° ± 25°. The 222 embryos examined on the nineteenth day were deducted in determining the number of 18-day embryos. Accordingly, the figures record the incidence of chicks dead in position II among embryos alive at 18 days and given the opportunity to hatch. The inevitable slight error from the probability that some of those in that malposition had hatched is distributed equally to the two groups and therefore does not prejudice the data.

TABLE 7.—*Effect of the position of the incubating egg upon the incidence of position II in embryos originally oriented with the head toward the small end and in those oriented otherwise*

Position during incubation	Total embryos at 4 days	Embryos oriented in the given class		Embryos alive at 18 days	Embryos subsequently dead in position II		Difference between percentages dead in position II in horizontal and tilted positions
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	
Embryos originally oriented 90°±37.5°, right:							
Horizontal	2,049	274	13.4	210	19	9.05	7.14±2.19
Tilted	1,815	239	13.2	209	4	1.91	
Embryos in all other orientations							
Horizontal	2,049	1,775	86.6	1,388	23	1.66	0.12±0.47
Tilted	1,815	1,576	86.8	1,432	22	1.54	

The percentages of the total number of embryos which were oriented toward the small end do not differ significantly in the horizontal and tilted eggs. This is to be expected if the orientation is established in early stages of cleavage. The fact that the expectation was realized indicates that the method used has permitted the determination of the original orientation at a stage before it might be concealed by movement of the embryo or modified by the position of the incubating egg.

Among all the embryos not originally oriented within the sector bounded by radii at 37.5° right and left of the small end, there is no significant difference between the frequencies of position II in the horizontal and tilted eggs. These embryos constitute 87 percent of those alive at the eighteenth day and are sufficient in number to reveal even a small difference between the horizontal and tilted positions if there were one. On the other hand, among those originally oriented within 37.5° of the small end, the frequency of position II is nearly five times as great in the eggs incubated in a horizontal position as in the tilted ones. The difference—7.14 percent—is 3.3 times its standard error and therefore statistically significant even in the relatively small numbers available for comparison.

On the basis of this analysis and of the data in table 5 it seems safe to conclude that for all embryos surviving to 18 days there is some risk of subsequently dying in position II and that for about 87 percent of them that risk is only slightly lessened, if at all, by incubation in the tilted position. Table 5 indicates that this risk is smallest for those not originally deviating more than 15° from the normal orientation.

The chances of death in the head-in-small-end malposition after the eighteenth day were approximately as follows for embryos in the different classes of orientation at 4 days or earlier in the writers' material:

For embryos at 0° ± 22.5° (normal), 1 per 100.
For other embryos, not within 37.5° of small end, 1.6 per 100.
For those at 90° ± 37.5° right of normal, if tilted, 2 per 100.
For those at 90° ± 37.5° right of normal, if horizontal, 9 per 100.

Hutt and Pilkey (*7*) pointed out that one way to reduce the mortality attributable to this malposition would be to incubate the eggs in the tilted position with the large end up during the critical period when the embryo is becoming fixed in its position with relation to the long axis of the egg. From the studies of Byerly and Olsen (*2*) this period would appear to be during the second week of incubation, while Kuo (*9*) terminates it by the eleventh day. The analysis given above shows why such treatment would be effective.

DISCUSSION

The frequent failure of chick embryos to attain the normal position for hatching undoubtedly accounts for the peak of mortality which occurs during the last few days of incubation. The head-in-small-end malposition (fig. 3, B), which has been shown in this study to result in some cases from incorrect orientation, places the embryo at a double disadvantage. With its head in the small end of the egg there is usually much less room for the embryo to work while initiating and carrying out the process of hatching. Moreover the air cell is inaccessible as a source of air to supplement the allantoic respiration before the shell is pipped.

Evidence has been gathered at this laboratory which shows that position II is likely to be fatal in the majority of cases, but on the other hand, a few chicks in this malposition have been definitely observed by the writers to hatch. It would appear, therefore, that though th head-in-small-end position is usually fatal, it is not so invariably. This is not in accord with the opinion of Réaumur (*11*), who noted the malposition as early as 1751 but did not consider it a handicap.

Although position II seems to be of relatively infrequent occurrence when the total number of fertile eggs is considered, it accounts for no small portion of the mortality in eggs incubated in a horizontal position. Over 18 percent of 5,050 embryos dying on or after the eighteenth day in eggs so incubated were found by Hutt (*6*) to be in position II. From the data in table 7, which indicate that at least 19 out of 42 cases of position II in horizontal eggs resulted from abnormal orientation, it would appear that about 45 percent of such deaths, or about 8 percent of the mortality in the last 4 days of incubation, is traceable in horizontal eggs to abnormal orientations. This amounts to a little less than 4 percent of the total mortality. From the data in table 7 it would appear that for tilted eggs the mortality from this cause is considerably less than this figure.

Successive readings of orientation in the present study have shown that the extra-embryonic blood vessels and the yolk have a relatively constant relationship to the long axis of the egg from 72 to 96 hours, and that this relationship is maintained in most cases until the end of the sixth day. During this period the vitelline blood vessels serve to anchor the embryo in a fixed position upon the yolk, and the chalazae, in turn, maintain the yolk in a fairly constant relationship to the long axis of the egg. Kuo (*9*) points out that the restraint exercised upon the movements of the embryo by these two factors persists in varying degrees up to the tenth day of incubation. This is undoubtedly why some of the embryos originally directed toward the small end are never able to escape from that initial handicap. On the other hand, after the fifth day the embryo moves with increasing freedom up to the tenth day or slightly later (Kuo). This, in turn, explains why, in the writers' material, 98 percent of 209 embryos originally oriented within 37.5° of the small end were subsequently able to assume the normal head-in-large-end position in eggs incubated in the tilted position. Evidently the freedom of movement which permits such a shifting is somewhat more restricted in the eggs incubated in the horizontal position.

Further tests will be necessary to determine whether or not the occurrence of position II independently of the original orientation can be overcome by such manipulations as multiple turnings or turning the eggs in more than one plane during the critical second week when the head-in-small-end position is apparently established.

It is particularly interesting that the orientation associated with the lowest total mortality rate should also be that in which the greatest number of embryos is found (table 3). The rates 11.1 and 23.7 percent in embryos oriented at 135° ± 22.5° left and 135° ± 22.5° right are based on small numbers, and can therefore hardly be compared with the others which are based on over 100 embryos in each class. Apart from these two classes, the embryos not deviating more than 22.5° to right or left of the normal position have the lowest rate of mortality, one which was earlier shown to be significantly lower than that for all other orientations combined. The numbers in the five largest classes seem amply large to rule out the possibility of this being a coincidence, but further data bearing on this point are desirable.

The special interest lies in the probability that the orientation at right angles to the long axis of the egg, and with the head to the left when the small end of the egg is directed away from the observer, has become the normal and the modal orientation because of its survival value. Such an explanation is tenable only if the angle of orientation of the embryo is in some measure an inherited character. Obviously it is not a character of the embryo, but rather one that is maternally determined. If of two hens one should produce eggs containing embryos oriented within a very narrow range from 0° ± 22.5°, and another yield embryos with a much wider scatter of orientations, or within a narrow range but one deviating from the normal, the progeny of the first would have a slight advantage over that of the second. If the tendency exhibited by the first hen were inherited it would be preserved by natural selection and would in time become established as the normal for the species. Unpublished data collected by the writers support this hypothesis.

SUMMARY

The orientation of 4,721 chick embryos was accurately determined by candling, without breaking the shell, at 84 to 90 hours of incubation. Seventy-five percent of these embryos lay within 45° right or left of a line at right angles to the long axis of the egg and with their heads to the left when the small end of the egg was directed away from the observer.

The mortality rate for embryos originally normally oriented was significantly lower than that for all the remaining embryos.

Abnormal early orientation had apparently no markedly adverse effect upon the viability of the embryo until the last 4 days of incubation.

One malposition of the fully formed embryo, that in which the body is upside down with the head in the small end of the egg, was five times as frequent among embryos which had been oriented toward the small end of the egg on the fourth day as among those then normally oriented.

When eggs containing embryos originally oriented within 37.5° of the small end of the egg were incubated in a tilted position with the large end up, the frequency of the head-in-small-end malposition was reduced to 1.9 percent, but among such eggs incubated in a horizontal position its frequency was 9 percent. Tilting the eggs did not materially reduce the frequency of this malposition among embryos having other initial orientations.

Four other malpositions appeared to be independent of the original orientation of the embryo.

It is suggested that the normal orientation of the chick embryo has been evolved and established by virtue of the greater survival value which it confers upon the embryo.

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COLORADO AND VIRGINIA STRAINS OF CODLING MOTH IN RELATION TO THEIR ABILITY TO ENTER SPRAYED AND UNSPRAYED APPLES[1]

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INTRODUCTION

The amount of arsenical insecticide applied to control the codling moth (*Carpocapsa pomonella* L.) increased steadily in the leading apple-growing sections of the country until the amount of excessive spray residue on the fruit became a problem in the fall of 1925. Increased competition in the production of clean fruit and the concentration of orchards in certain areas, with the resulting increase in codling-moth population, have, doubtless, contributed to the necessity of increasing the number of sprays. The codling moth, however, continues as the major apple pest in spite of this. Just why this should be true is difficult to explain fully, but some of the possible factors involved are presented in this paper. The codling-moth material used in this study originated in two orchard districts having wide differences in requirements for control, namely, the Grand Valley of Colorado and the Shenandoah Valley of Virginia. A brief history of control requirements in each district is reviewed here.

An investigation of codling-moth control work in Virginia revealed that very few commercial apple orchards in the Shenandoah Valley were sprayed prior to 1900. According to pioneer growers the first orchard sprayer used in the Winchester district was soon after 1900. Up to 1915 the general practice was to apply one spray, the calyx application. In 1909 Quaintance and others (*10*)[3] demonstrated the efficiency of the calyx spray alone in controlling the codling moth in a number of orchards in the eastern part of the United States. Two of the orchards were in Virginia. From 1916 to 1920 the number of codling-moth sprays was increased to two, and in a very few instances to three, in the best-managed orchards. The author came to the Shenandoah Valley in 1921, a year when no sprays were applied because the apple crop was frozen. From 1922 to 1930 the number of applications was increased to four in the best-managed orchards. Because of economic conditions the number of sprays has not been increased since 1930.

A summary of the history of spraying for codling-moth control in the Grand Valley of Colorado was given to the writer by Dr. George List of the Colorado Agricultural Experiment Station in a letter dated December 8, 1928, as follows:

> The first accurate record of the codling moth in the Grand Valley was in 1891. At that time the moth was just getting well established and was attracting some

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[2] The author acknowledges the assistance of R. F. Hatcher, Mrs. A. B. Groves, Miller McDonald, Holmes Fries, and Earl Luttrell, who did much of the routine work involved in this study. Fig. 1 was photographed by Dr. A. B. Groves.

[3] Reference is made by number (italic) to Literature Cited, p. 552.

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attention. A few of the growers began to spray about that time, but spraying was not general for some three or four years.

For a number of years only the calyx application was made. In 1900 and 1901 it was generally recommended that one cover spray be applied in addition to the calyx spray. In 1904 two cover sprays were quite generally used and a few of the growers were applying a third. During all of this time the percentage of loss was quite low, as compared with present losses. From 2 to 5 percent of wormy fruit was considered a very heavy loss. In 1906 the general recommendation was for five applications, the calyx and four cover sprays.

In 1907 Dr. Gillette and E. P. Taylor carried on an experiment in an orchard in that section in which they used a number of combinations. They came to the conclusion that four sprays were all that was necessary. In spite of this, however, the number of applications continued to increase. In 1909, which was the year of my first experience in that section, the general practice was to make five or six applications. By 1914 the infestation continued to increase, and six or seven sprays were used by practically all of the growers. From 1914 to the present time we have had demonstration blocks practically every year, and we have been testing the number of applications as well as a number of other points. In spite of all the information we have been able to get and to give the growers, the number of applications has been increasing and the results have not been as good generally as in former years. During the last four or five years the growers have been applying from eight to ten sprays and as high as twelve is not uncommon.

The wide difference in requirements for control is usually attributed to the influence of such environmental factors as climate and natural enemies, or to other local "conditions." It has been shown (*6, 8, 11, 12*) that the seasonal history of the codling moth in the Grand Valley of Colorado and the Shenandoah Valley of Virginia is similar, even to the time of deposition of the various broods of eggs. A comparative study of the codling moth from the two valleys was undertaken at Winchester, Va., in 1927. It was hoped that information might be obtained concerning some of the reasons for the wide difference in requirements for control and in the number of sprays applied. Results of the first two season's work show (*4, 5*) that the Colorado larvae were distinctly more successful in entering apples sprayed with lead arsenate and that crosses between the Colorado and Virginia moths were intermediate between the parent strains with respect to their ability to enter the sprayed fruit.

From 1929 to 1933 studies were included to determine whether the apparent tolerance is specific for arsenic, and to observe the effect of the Virginia climate on the Colorado strain and crosses, also the effect of rearing larvae on sprayed fruit exclusively, and something concerning the nature of the differences already demonstrated. Unless stated otherwise, the variety of apple used was the Arkansas (Mammoth Black Twig).

Thorpe (*13*) has reviewed the literature on the subject of insect strains or races.

METHODS

In comparing the ability of the larvae to attack sprayed fruit, the apples were sprayed by the writer in all instances. The calyx end of each apple was covered with shellac, and the apples were then suspended by their stems for spraying. Two sprays were applied, the second application being made as soon as the first coating had dried. Attempts were made to coat the apples alike in each test, and the residue was considerably greater with the double than with a single spraying. As soon as the spray material dried, codling-moth eggs were placed on the apples (average of 10 per apple) in the manner already described and illustrated by Hough (*5*). Eggs of the same age were used for all strains in each experiment. The fruit was suspended in an insectary screened on all sides. The eggs hatched within 1 to 3 days after they were placed on the apples, and the fruit was examined 5 to 10 days after the eggs hatched.

Each year a fresh supply of lead arsenate was obtained for the experiments. The same commercial brand was used from 1927 to 1933, inclusive, except in 1929 when it was necessary to secure a fresh supply of another brand. The cryolite used in 1929 was a synthetic product whereas the cryolite used in 1933 was a natural product, processed for insecticide purposes.

Standard Weather Bureau maximum and minimum thermometers were used for recording temperature. A wet-and-dry-bulb hygrometer was used for humidity observations. Where larvae hatched in a saturated atmosphere the fruit was suspended in battery jars (gallon size), which contained about 2 inches of water. The top of each jar was closed with moisture-proof cellophane. When newly hatched larvae were confined in a saturated atmosphere the young worms were kept in closed Stender dishes containing a close-fitting disk of water-soaked blotting paper on the bottom.

A balance having a sensitivity of 0.01 milligram was used in weighing the larvae. One hundred newly hatched larvae were weighed at the same time. The larvae were collected in a straight-edged vial (one half inch in diameter and 3¼ inches high), a piece of cheesecloth was stretched over the open top and the vial placed in a cyanide bottle just long enough to render the larvae quiescent. This usually required about 20 minutes. Upon removal from the cyanide bottle and just before weighing, the larvae were carefully counted by placing one larva in each of 100 squares. When comparisons were to be made between strains, the work of collecting, preparing, and weighing each strain took place at the same time.

Granular potassium cyanide was used in preparing the cyanide bottles in which the newly hatched larvae were fumigated. Close-fitting disks of blotting paper kept the granules in the bottom. No water was added. Newly hatched larvae were collected in straight-edged vials as described above and fumigated for the desired period. With each fumigation the cyanide bottles were altered between the Colorado and Virginia larvae. In 1931 and 1932 pint fruit jars served as cyanide jars for fumigation of the eggs. In 1933 a large museum jar (35 by 30 centimeters) was used, so that all eggs of the various ages desired and of each strain could be fumigated simultaneously in the same chamber. To accomplish this, two circular wire baskets were made, each having six equal compartments. Twelve pear leaves or pieces of cellophane containing the eggs were placed in each of the 12 compartments, about one half inch of granular cyanide covered the bottom of the jar, and over the baskets there were four 4-inch Petri dishes filled with granular cyanide as shown in figure 1.

Second-instar larvae and mature larvae were fumigated in the museum jar just described. The larvae were first placed in Stender dishes, and each dish was covered with a fine-meshed wire gauze.

In the course of this investigation it was soon learned that dosages of insecticides (sprays or fumigants) could be made too great or too small to bring out any differences in the vitality of the individuals under test. When the dosage was too great, the kill approached 100 percent for all strains. When the dosage was too small, the proportion of survival was so nearly the same for all strains as to mask any differences in their vitality. Correct dosage is absolutely essential in a comparative study of this kind.

DESIGNATION OF STRAINS

The Colorado strain consisted of larvae received October 24, 1927, from Grand Junction, Colo. Each generation was reared on unsprayed apples or on apples which carried only a very small amount of spray material.

The Colorado-K strain was received from Grand Junction November 12, 1928. Each generation was reared on apples sprayed with lead arsenate just before the fruit was placed in the rearing jars.

The Virginia strain was from native stock. Each generation was reared on unsprayed apples or on apples which carried only a very small amount of spray material. Larvae of this lot and the Colorado strain and their crosses were always reared on fruit which carried very little or no insecticide.

The Virginia-K strain also came from native stock. Each generation since July 1, 1929, was reared on apples sprayed with lead arsenate just before the fruit was placed in the rearing jars.

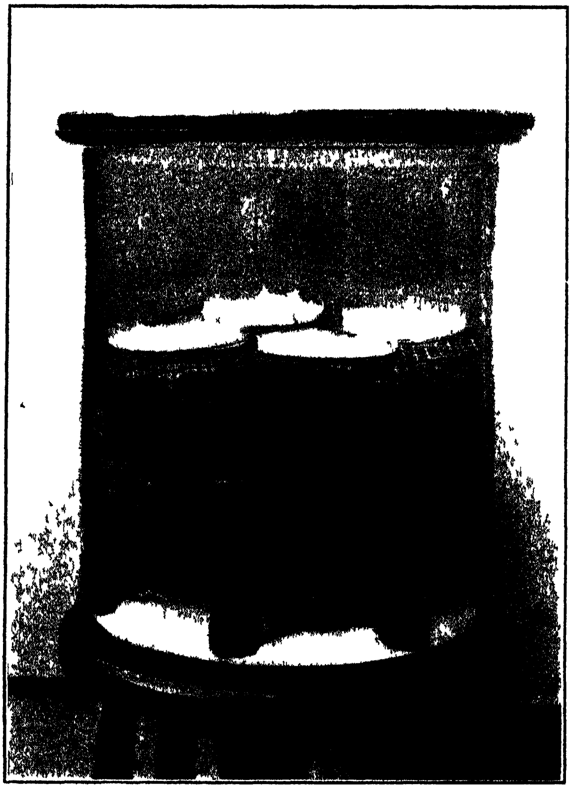

FIGURE 1 – Fumigation jar showing method of fumigating eggs on leaves placed in circular wire baskets of six compartments each.

The Colorado-Virginia crosses were the progeny of crosses made with moths in July 1928. The F_1 generation was reared in 1928, the F_2 and F_3 generations in 1929, and so on, ending with F_{10} generation in June 1933.

The fruit used for the Colorado-K and Virginia-K larvae was first placed in a single layer on large wire trays (2 by 6 feet). A bucket sprayer was used to apply the lead-arsenate spray to all sides of the fruit until dripping began. For the Colorado-K larvae the fruit received lead arsenate at the rate of 1½ pounds per 100 gallons in the early years of the work, but this was finally increased to 4 pounds. For the Virginia-K larvae lead arsenate was used at the rate of 1 pound and finally increased to 2 pounds per 100 gallons.

EXPERIMENTAL RESULTS

STRAINS

At the close of the work in 1928 the question naturally arose as to whether the demonstrated difference between Colorado and Virginia larvae was specific only for arsenic. In 1929 such nonarsenicals as cryolite, barium fluosilicate, and rotenone were used in comparative tests with lead arsenate. The results (table 1) show that the Colorado larvae were distinctly more successful in entering fruit sprayed with any of the insecticides used. Of course, it was possible to use a material at such a strength that the difference was small as in the case of experiment 11 in which barium fluosilicate was used twice at the rate of 8 pounds per 100 gallons. Eggs placed on the apples sprayed with rotenone hatched 3 days after the spray was applied; and in the case of the other materials the eggs hatched within 1 to 3 days after the application of the sprays.

In experiment 7 the larvae designated as Colorado-K were from eggs deposited by the spring brood of moths, which came from larvae received from Grand Junction, Colo., on November 12, 1928. It appears that the Colorado-K and Colorado larvae were alike with respect to their ability to enter fruit sprayed with lead arsenate. Henceforth, the Colorado-K larvae were reared on fruit sprayed with lead arsenate a short time before the larvae hatched.

During the season of 1929 as well as in all succeeding seasons it was difficult to account completely for the differences in the percentage of control obtained. Temperature at the time of hatching of the larvae, variation in the spray covering, and the variety of apples used in different experiments may have contributed to the differences. Sometimes it seemed that the spray adhered to the fruit better than at other times, in spite of the fact that the applications were made as nearly alike as possible. Humidity or slight air movements at the time of spraying might have contributed somewhat, but the chief cause seemed to be the result of variations in the wax coating of the fruit. Markley and Sando showed (*9*) that the apple skin undergoes progressive changes in constituents and physical properties during growth. Ursolic acid and oil increase in amount, with oil increasing more rapidly as the apple approaches maturity. Furthermore, they showed that the apple skin on the sunny side contains larger quantities of oil than that on the shady side of the same fruit, while the opposite is true for ursolic acid content. In picking apples for the experiments reported herein no attention was given to the part of the tree from which the apples came. It follows that there must have been variations in the oil content of the skin of the fruit used.

In considering the factors influencing variations in control as recorded in table 1, the average number of eggs placed on each apple was 10 and the percentage of hatch was approximately the same for each strain in a given test. The number of apples used, however, was not always the same for each strain in a given test and the total number of apples used in each experiment was not always the same.

During the season of 1930 the lead arsenate tests were repeated in experiments 12 and 13 to learn whether any important change had taken place in the comparative ability of the Colorado and Virginia larvae to enter sprayed fruit. No important change was apparent in the results obtained. The results of similar experiments in 1932 and 1933 also failed to indicate any important change in either strain.

TABLE 1.—*Comparative tests with Colorado, Virginia, Colorado-K, and Virginia-K codling-moth larvae on apples which received 2 applications of an arsenical or a nonarsenical spray*

Experiment no.	Date	Apple variety	Strain	Check, unsprayed					Lead arsenate, 4 pounds in 100 gallons					Cryolite, 4 pounds in 100 gallons					Barium fluosilicate, 4 pounds in 100 gallons					Rotenone, 1 g in 2,000 ml				
				Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries	
				No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.
7	May 25, 1929	Early harvest	Colorado	483	428	88.6	430	89.0	555	191	34.4	350	63.0	588	208	35.3	356	60.5										
			Virginia	519	404	77.8	412	79.3	583	16	2.7	197	33.7	721	158	21.9	380	52.7										
			Colorado-K						565	191	33.8	348	61.5	436	152	34.8	293	67.2										
8	June 6, 1929	Arkansas	Colorado	574	514	89.5	516	89.8	620	243	39.1	377	60.8	610	261	42.7	400	65.5										
			Virginia	571	457	80.0	460	80.5	723	59	8.1	316	43.7	685	169	24.6	360	52.5										
			Colorado											[a]583	[a]169	[a]28.9	[a]278	[a]47.6										
			Virginia											[a]643	[a]108	[a]16.7	[a]270	[a]41.9										
9	July 17, 1929	York imperial	Colorado						486	125	25.7	243	50.0															
			Virginia	395	234	59.2	250	63.2	463	3	.6	74	15.9															
11	Aug. 2, 1929	do.	Colorado	536	452	84.3	452	84.3	711	104	14.6	316	44.4	[a]634	[a]162	[a]25.5	[a]328	[a]51.7	459	46	10.0	185	40.3	377	57	15.1	67	17.7
			Virginia	605	390	64.4	388	64.1	803	2	.2	244	30.3	[a]656	[a]44	[a]6.7	[a]236	[a]35.9	592	20	3.3	184	31.0	440	24	5.4	25	5.6
			Colorado																[a]559	[a]24	[a]4.2	[a]209	[a]37.3					
			Virginia																[a]593	[a]2	[a]0.3	[a]189	[a]31.8					
12	June 11, 1930	Arkansas	Colorado	405	351	86.7	361	89.1	582	119	20.4	307	52.7															
			Virginia	344	232	67.4	244	70.9	547	23	4.2	175	31.9															
13	Sept. 4, 1930	York imperial	Colorado						177	39	22.0	83	46.9															
			Virginia	357	265	74.2	273	76.4	364	11	3.0	78	21.4															
18	June 25, 1932	Arkansas	Colorado	512	374	73.0	422	82.4	783	134	17.1	343	43.8															
			Virginia	630	421	66.8	498	79.0	710	10	1.4	165	23.3															
19	July 23, 1932	do.	Colorado	200	150	75.0	155	77.5	348	63	18.1	153	43.9															
			Virginia	230	125	54.3	135	58.6	319	8	2.5	75	23.5															
			Colorado-K						602	284	47.2	390	64.8															
			Virginia-K						380	62	16.3	137	36.0															
20	Aug. 1, 1932	do.	Colorado	219	186	84.9	205	93.6	393	121	30.8	218	55.5															
			Virginia	245	159	64.9	193	78.8	325	4	1.2	61	18.7															
			Colorado-K						648	271	41.8	402	62.0															
			Virginia-K						460	88	19.1	228	49.5															
21	June 7, 1933	do.	Colorado	343	307	89.5	314	91.5	417	76	18.2	160	38.3															
			Virginia	347	302	87.0	314	90.4	416	16	3.8	125	30.0															
			Colorado-K						389	116	29.8	194	49.8															
			Virginia-K						403	54	13.3	128	31.7															
22	June 8, 1933	Baldwin (?)	Colorado	405	359	88.6	364	89.8	439	133	30.2	268	61.0															
			Virginia	378	292	77.2	310	82.0	421	35	8.3	153	36.3															
			Colorado-K						866	328	37.8	492	56.8															
			Virginia-K						927	162	17.4	435	46.9															

																			Bentonite nicotine,[b] 6 pounds, and nicotine sulphate, 1 pint in 100 gallons									
23	July 28, 1933	Arkansas	Colorado	333	263	78.9	274	82.2	401	94	23.4	188	46.8	[c]434	[c]100	[c]23.0	[c]211	[c]48.6	423	41	9.6	60	14.1					
			Virginia	315	224	71.1	242	76.8	417	29	6.9	134	32.1	[c]439	[c]58	[c]13.2	[c]165	[c]37.5	450	19	4.2	29	6.4					
			Colorado-K						436	183	41.9	248	56.8	[c]453	[c]175	[c]38.6	[c]276	[c]60.9	476	65	13.6	80	16.8					
			Virginia-K						425	93	21.8	227	53.4	[c]393	[c]81	[c]20.6	[c]209	[c]53.1	437	38	8.6	49	11.2					
24	Aug. 5, 1933	do	Colorado	278	216	77.6	228	82.0											413	28	6.7	60	14.5					
			Virginia	295	212	71.8	221	74.9											372	16	4.3	22	5.9					
			Colorado-K																382	57	14.9	83	21.7					
			Virginia-K																377	39	10.3	55	14.5					
																			Cuprous cyanide, 3 pounds in 100 gallons									
25	Aug. 11, 1933	do	Colorado	308	236	76.6	252	81.8	401	90	22.4	186	46.3	423	69	16.3	190	44.9	388	143	36.8	270	69.5					
			Virginia	290	179	61.7	217	74.8	386	17	4.4	87	22.5	392	19	4.8	117	29.8	402	81	20.1	220	54.7					
			Colorado-K						142	165	37.3	240	54.2	411	62	15.0	190	46.2	383	183	47.7	270	70.4					
			Virginia-K						395	54	13.6	166	42.0	394	36	9.1	150	38.0	388	103	26.5	221	56.9					
26 and 27	Aug. 17 and 21, 1933	do	Colorado	874	716	81.9	782	89.4	846	143	16.9	436	51.5															
			Virginia	817	601	73.5	668	81.7	798	15	1.8	174	21.8															
			Colorado-K						807	177	21.9	411	50.9															
			Virginia-K						766	96	12.5	324	42.2															

[a] Insecticide used at the rate of 8 pounds in 100 gallons.

[b] Contained 5 percent nicotine

[c] Insecticide used at the rate of 2 pounds in 100 gallons.

After rearing Colorado-K larvae on sprayed fruit through 7 generations and Virginia-K larvae through 6 generations, the eighth generation of Colorado-K and the seventh generation of Virginia-K larvae were included in tests made in July and August 1932. The results are given in experiments 19 and 20 (table 1). Colorado-K larvae were more efficient than Colorado larvae in their ability to enter and injure the fruit. A surprising difference was shown between Virginia-K and Virginia larvae, the former being distinctly more successful in entering the sprayed apples. Colorado larvae continued to demonstrate their superiority over Virginia larvae in their ability to enter sprayed fruit.

In the six experiments of 1933 (table 1) the order of success with which the larvae entered fruit sprayed with lead arsenate was Colorado-K, Colorado, Virginia-K, and lastly Virginia. The same order was also maintained in all tests with nonarsenical insecticides, except with nicotine in experiment 24 and cryolite in experiment 25. The fact that the Colorado-K strain did not exceed the Colorado strain in percentage of live larvae in experiment 25 might have been due to a slightly heavier coating of cryolite on the apples attacked by the Colorado-K larvae. In experiment 24 as well as in experiment 23 the heavy deposit of nicotine tended to obliterate marked differences between the strains. In these experiments the larvae hatched 2 days after the apples were sprayed with nicotine.

Three sets of crosses were made and the results of tests with the progeny of each were previously reported as shown in table 2. The first and second sets were discontinued after the F_2 generation of each cross was included in the tests of 1928. All subsequent experiments were made with the progeny of the third set of crosses and the results of these experiments are given in table 3.

TABLE 2.—*Crosses of the Colorado and Virginia codling moths*

Set of crosses	Crosses made	Previously reported tests with each generation			
		Experiment no.	Date of experiment	Generation	Reference [a]
First	June 1927	2	July 26, 1927	F_1	(4)
		3	Aug. 10, 1927	F_1	(4)
		4	June 8, 1928	F_2	(5)
		5	June 22, 1928	F_2	(5)
Second	May 1928	4	June 8, 1928	F_1	(5)
		6	Aug. 9, 1928	F_2	(5)
Third	July 1928	6	do.	F_1	(5)

[a] Reference is to Literature Cited

In 5 of the 8 tests recorded in table 3 the cross Colorado female × Virginia male was more successful in entering the sprayed fruit than was the cross Virginia female × Colorado male. If the totals of the 8 tests are considered, the results are as follows: Colorado female × Virgina male 3,942 eggs hatched, 368 live larvae or 9.3 percent; Virginia female × Colorado male, 4,202 eggs hatched, 330 live larvae or 7.8 percent. Both crosses maintained an intermediate position between the parent strains in the proportion of larvae which entered the fruit. Table 1 gives the results for the parent strains in the same experiments, which may be identified by the experiment number. Parent strains were not included in experiments 10 and 11a of 1929 and the Colorado strain was not included in experiment 15 of 1931.

TABLE 3.—*Comparative tests with the F_3 and succeeding generations of the crosses of the Colorado and Virginia codling moths on apples which received 2 applications of spray at the rate of 4 pounds of lead arsenate to 100 gallons of water*

Experiment no., date, and apple variety	Cross and generation	Eggs hatched	Live larvae		Total injuries	
		Number	*Number*	*Per cent*	*Number*	*Per cent*
9, July 17, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_3	510	30	5.8	199	39.0
	Colorado ♀ × Virginia ♂ F_3	487	16	3.2	176	36.1
10, July 25, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_3	556	40	7.1	208	37.4
	Colorado ♀ × Virginia ♂ F_3	576	53	9.2	229	39.7
11 a, Aug. 12, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_3	485	14	2.8	140	28.8
	Colorado ♀ × Virginia ♂ F_3	503	30	5.9	149	29.6
12, June 11, 1930, York Imperial	Virginia ♀ × Colorado ♂ F_4	632	54	8.5	280	44.3
	Colorado ♀ × Virginia ♂ F_4	395	58	14.7	184	46.6
13, Sept. 4, 1930, York Imperial	Virginia ♀ × Colorado ♂ F_6	482	37	7.7	166	34.4
	Colorado ♀ × Virginia ♂ F_6	468	65	13.8	182	38.8
15, July 23, 1931, Arkansas	Virginia ♀ × Colorado ♂ F_7	701	33	4.7	283	40.4
	Colorado ♀ × Virginia ♂ F_7	637	48	7.5	272	42.7
21, June 7, 1933, Arkansas	Virginia ♀ × Colorado ♂ F_{10}	416	51	12.2	149	35.8
	Colorado ♀ × Virginia ♂ F_{10}	421	43	10.2	133	31.5
22, June 8, 1933, Baldwin (?)	Virginia ♀ × Colorado ♂ F_{10}	420	71	16.9	216	51.4
	Colorado ♀ × Virginia ♂ F_{10}	455	55	12.0	204	44.8

In all the work with the three sets of crosses 15 comparative tests were made from 1927 to 1933. In 7 of the experiments the cross of Virginia female × Colorado male showed a slightly higher percentage of live entrants, whereas the Colorado female × Virginia male was slightly more successful in 8 of the tests. Each cross showed a slightly higher percentage of live entrants in 2 of the 4 tests with larvae from the first set of crosses. The Virginia female × Colorado male was slightly more successful in both experiments which included larvae from the second set of crosses. In the third set, the Colorado female × Virginia male showed a higher percentage of live larvae in 6 of the 9 tests, 8 of which were made between 1929 and 1933 and are given in table 5. It is not clear that one cross was superior to the other in its ability to enter the sprayed fruit. It is clear, however, that both crosses were superior to Virginia larvae but inferior to Colorado larvae in this respect.

BACK CROSSES

In the spring of 1931 Colorado and Virginia moths of both sexes were crossed with moths of the F_5 generation of the cross Virginia female × Colorado male. Larvae of the F_1 and F_2 generations of back crosses were included in tests with Colorado and Virginia larvae and the F_7 generation of crosses. The results, given in table 4, show that crossing back with Colorado moths increased the ability of the larvae to enter sprayed fruit, while crossing back with Virginia moths did not. In fact, larvae of the F_2 generation of back crosses with Virginia codling moths were not so successful in entering sprayed fruit as were the crosses, and were most like Virginia larvae in this respect. It is probable that continued back crossing with the parent strains would have restored the Colorado and Virginia types.

In experiment 14, larvae of the back cross with Colorado males exceeded the Colorado strain in the percentage of live entrants. In the F_2 generation, however, the Colorado larvae were more successful. The back cross with Colorado females appeared slightly less successful in the F_1 generation than the back cross with Colorado males. In the F_2 generation, however, the back crosses were more nearly alike in their ability to enter the sprayed fruit.

In experiment 16 the percentage of live larvae was reduced for all larvae including the checks. It is thought that the chief cause of the reduction was the extra amount of lead arsenate on the fruit. The fruit was of the Arkansas variety as in the other tests, but the apples received an application of lead arsenate in the orchard about 3 weeks prior to the experiment.

TABLE 4.—*Comparative tests with Colorado and Virginia codling-moth larvae, the F_7 generation of crosses and F_1 and F_2 generations of back crosses on apples which received two applications of spray at the rate of 4 pounds of lead arsenate to 100 gallons of water, 1931*

	Experiment 14, June 13, F_1 of back cross			Experiment 15, July 23, F_2 of back cross			Experiment 16,[a] Aug 3, F_2 of back cross		
	Eggs hatched	Live larvae		Eggs hatched	Live larvae		Eggs hatched	Live larvae	
BACK CROSS	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Virginia ♀ } ♀ F_5 Colorado ♂ } Colorado ♂	547	129	23.6	829	117	14.1	394	31	7.9
Colorado ♀ Virginia ♀ } ♀ F_5 Colorado ♂ }	469	80	17.0	849	109	12.9	439	35	8.0
Virginia ♀ } ♀ F_5 Colorado ♂ } Virginia ♂	553	38	6.9	862	18	2.0	386	5	1.3
Virginia ♀ Virginia ♀ } ♂ F_5 Colorado ♂ }	473	35	7.4	913	31	3.4	455	8	1.7
STRAIN AND GENERATION OF CROSS									
Colorado	625	128	20.5	-	-	-	439	71	16.2
Virginia	499	9	1.8	645	12	1.9	433	3	6
Virginia ♀ } F_7 Colorado ♂ }	-	-	-	701	33	4.7	376	10	2.6
Colorado ♀ } F_7 Virginia ♂ }	-	-	-	637	48	7.5	393	20	5.0
Colorado (check)	-	-	-	-	-	-	386	224	58.0
Virginia (check)	-	-	-	-	-	-	399	72	18.0

[a] Apples used in experiment 16 were sprayed by mistake with lead arsenate in the orchard during week of July 13

SECOND-INSTAR LARVAE

Observations in June 1933 on the length of the first instar were made by placing 31 newly hatched Virginia larvae on thick slices of apples, which were kept in closed Stender dishes to conserve moisture. The larvae were removed soon after molting. The length of the first instar ranged from 3.5 to 7 days, averaging 4 days. Jenne (7) found the average length of the first instar to be 5.6 days (range 4 to 7 days) for 12 individuals, but he concluded that handling the larvae daily or every 2 days in order to place them on fresh pieces of apple caused the larvae to develop slowly.

Colorado and Virginia larvae of the second instar ranging in age from 5 to 7 days were taken out of unsprayed fruit in the insectary and placed on sound apples. Although the second-instar larvae were not nearly so active as newly hatched larvae, table 5 shows slightly more than 60 percent of the Colorado larvae and a fraction more than 48 percent of the Virginia larvae were successful in entering unsprayed apples. On sprayed fruit, however, entry was very difficult, and it was necessary to reduce considerably the coverage of lead arsenate as compared with the coverage used for newly hatched worms in order to obtain a fair proportion of survival of both strains. The second-instar larvae were very sluggish when placed on sound apples and were inactive much of the time. Some fell from the fruit, others lost moisture and died, while others finally entered the apples. The Colorado larvae of the second instar appeared to be somewhat more successful in entering both sprayed and unsprayed fruit.

TABLE 5.—*Comparative tests*[a] *with Colorado and Virginia codling-moth larvae of the second instar on apples sprayed with lead arsenate, 1933*

Experiment no., date, and apple variety	Quantity of lead arsenate per 100 gallons	Strain of larvae	Age of larvae	Total larvae	Live larvae		Total injuries	
			Days	*Number*	*Number*	*Percent*	*Number*	*Percent*
1, June 29, Arkansas	2 pounds, 1 spray	Colorado	6	300	64	21.3	71	24.6
		Virginia	6	300	30	10.0	52	17.3
	Check (not sprayed)	Colorado	5–6	200	126	63.0	130	65.0
		Virginia	5–6	200	97	48.5	104	52.0
2, June 30, Arkansas	1 pound, 1 spray	Colorado	6–7	200	72	36.0	80	40.0
		Virginia	6–7	200	22	11.0	28	14.0
	Check (not sprayed)	Colorado	6	100	59	59.0	63	63.0
		Virginia	6	100	48	48.0	51	51.0
3, Aug. 7, Arkansas	3 pounds, 2 sprays	Colorado	7	123	4	3.2	42	34.1
		Virginia	7	139	0	0	9	6.4
4, Aug. 8-12, Arkansas	2 pounds, 1 spray	Colorado	7	408	49	12.0	207	50.7
		Virginia	7	427	33	7.7	139	32.5

[a] In experiments 1 and 2 larvae were placed on the fruit suspended from poles in the usual manner. In experiments 3 and 4 larvae were placed on the apples in battery jars.

EFFECT OF TEMPERATURE ON LARVAE

In the early years of the work it was observed that a lower percentage of larvae usually entered the sprayed and unsprayed apples in an experiment, when the weather was cool during the period of hatching. Table 6 shows the relation between the temperature and the percentage of successful entrants found in unsprayed Arkansas apples. When the mean temperature ranged from 59° to 69° F. and the maximum from 70° to 82°, 62.4 percent of the Virginia larvae and 79.9 percent of the Colorado larvae entered the fruit; but when the mean temperature ranged from 70° to 82° and the maximum from 82° to 98°, the successful entrants increased to 76.3 percent for the Virginia larvae and 86 per cent for the Colorado larvae. A careful examination of percentage of live larvae in the sprayed fruit in the same experiments shows a somewhat similar effect. Cutright (*3*), using controlled temperatures, showed that the increase of temperatures within seasonal ranges aids the codling-moth larvae in establishing themselves in sprayed and unsprayed apples. The data given in table 8 must be taken into consideration when accounting for variations obtained in the experimental results. Attention is also called to the consistently higher percentage of Colorado larvae as compared with the percentage of Virginia larvae found in the unsprayed apples in the various experiments.

Table 6.—*Relation between temperature and the number of successful entrants of Colorado and Virginia codling-moth larvae on unsprayed Arkansas apples which were used as checks in the various experimental tests*

Experiment no	Year	Temperature during time of hatching		Virginia strain			Colorado strain		
		Mean	Maximum	Larvae	Live larvae		Larvae	Live larvae	
		°F	°F	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
	1928	59	71	470	253	53.8		---	
	1932	66	70	455	319	70.1	429	370	86.2
	1928	67	77	480	295	61.4	438	369	84.2
	1928	69	82	293	186	63.4	398	299	75.1
	1932	69	76	230	125	54.3	200	150	75.0
	1933	69	80	295	212	71.8	278	216	77.6
	1933	69	80	290	179	61.7	308	236	76.6
Total				2,513	1,569	62.4	2,051	1,640	79.9
	1929	70	90	571	457	80.0	574	514	89.5
	1933	71	82	328	297	90.5	395	352	89.1
	1930	72	85	344	232	67.4	405	351	86.7
	1933	72	88	427	304	71.1	444	364	81.9
	1932	78	89	270	196	72.6	216	173	80.0
	1932	78	88	245	159	64.9	219	186	81.9
	1933	80	98	378	292	77.2	405	359	88.6
	1933	81	93	315	224	71.1	333	263	78.9
	1933	82	98	317	302	87.0	343	307	89.5
Total				3,225	2,463	76.3	3,334	2,869	86.0

REJECTION OF POISONED MATERIAL BY LARVAE

Early in the investigation it was suggested that possibly the Colorado larvae rejected more of the poisoned part of the apple in entering the fruit. In 1930 a careful study was made of Colorado and Virginia larvae as they worked to enter the fruit. Two small pits about 1 millimeter square were cut side by side on the surface of an apple. A drop of a solution of arsenic acid was placed in each pit. In all but the last series the solution was stained with gentian violet to enable the observer to see where the poison had dried. A recently hatched Colorado larva was placed in one pit and a Virginia larva in the other. Both were viewed simultaneously in the same field of the binocular microscope.

In 20 minutes many of the larvae had buried themselves to such an extent that thereafter it was difficult to make accurate records of rejections. Others had quit on account of the action of the poison taken into the alimentary tract. The actual number of times each larva was seen to bite off a piece of apple pulp and throw it away was recorded. The totals for each series of observations and all observations are given in table 7. The larvae that did nothing in the first 20 minutes were not included. Three of these were Colorado and two Virginia larvae. None of the larvae was dead at the end of 20 minutes. A number appeared sick and had quit work, but it was impossible to conclude from the number of rejections made in cutting through the poisoned walls of the apple pits which larvae were likely to die or live. There was no difficulty in seeing each rejection made following the bite cut loose from the apple. It appears that the poison each larva takes into its system is accidental and comes from adherence of minute amounts of poison to the mandibles. The poison may later be carried into the alimentary tract when the larva feeds. The larva's first concern is to bury itself and then feed, but in burying itself it cuts through and rejects the surface of the apple where the poison is found.

TABLE 7.—*Rejections of apple pulp made by newly hatched Colorado and Virginia codling-moth larvae during the first 20 minutes after being placed in pits poisoned with a solution of arsenic acid, 1930*

Series no.	Dilution of As_2O_5	Colorado strain						Virginia strain					
		Larvae		Rejections in 20 minutes				Larvae		Rejections in 20 minutes			
		Alive	Dead	Maximum	Minimum	Total	Average	Alive	Dead	Maximum	Minimum	Total	Average
1	1 to 5,000	10		42	3	175	17.5	7		33	1	110	15.7
			0						1			31	31.0
2	1 to 1,000	7		29	2	112	16.0	6		35	1	76	12.6
			2	19	5	24	12.0		4	21	9	64	16.0
3	do	9		49	14	261	29.0	5		44	6	129	25.8
			1			16	16.0		5	33	3	100	20.0
4	do	8		52	12	214	26.7	8		45	7	229	28.6
			2	20	7	27	13.5		2	31	8	39	19.5
5	do	6		70	4	185	30.8	5		63	10	176	35.2
			3	33	14	62	20.6		5	13	14	123	24.6
6	do	5		33	8	85	17.0	2		27	2	29	14.5
			4	11	6	36	9.0		8	50	7	186	23.2
Total (alive)		45				1,032	22.9	33				749	22.6
Total (dead)			12			165	13.7		25			543	21.7
Percent		78.9	21.1					56.9	43.1				

Table 7 shows that the 12 Colorado larvae which finally died averaged 13.7 rejections per larva as compared with 21.7 rejections per larva for the 25 dead Virginia larvae. In other words, the Colorado larvae were fatally poisoned after biting off a fewer number of pieces and rejecting them, but only 21 percent of the Colorado larvae died as compared with 43.1 percent of the Virginia larvae. The average number of rejections per larva made by the live larvae was practically the same for both strains.

WEIGHT OF LARVAE

Campbell (*1*) working with silkworms showed that the minimum lethal dose of arsenic varied directly with the weight of the insect and could be expressed per unit weight or mass of insect. It was thought that a comparison of the weights of young codling-moth larvae of the various strains might yield some information that would aid in explaining the observed differences in the ability of the larvae to enter sprayed and unsprayed apples. By weighing 100 larvae at a time it was found (table 8, series A, B, C) that all the larvae weighed distinctly less when the temperature was high and the humidity relatively low as in series B. In series A and C the percentage of atmospheric humidity was about the same for each series, but the saturation deficiency of the air and, therefore, the evaporation was probably greater for series C, because the temperature was higher. It will be observed the larvae of each strain in series C weighed slightly less than those of the corresponding strain in series A. Virginia larvae under atmospheric conditions weighed less than Colorado larvae, but the weight of the cross Virginia female × Colorado male in series A exceeded the weight of the Colorado larvae, and in series B, the cross Colorado female × Virginia male was equal in average weight to the Colorado larvae. In other words, there was no evidence of any relation between weight of larvae and the comparative ability of the strains and the crosses to enter sprayed fruit.

TABLE 8.—*Weight of young codling-moth larvae of the Colorado and Virginia strains as determined by weighing 100 larvae at each weighing*

Series	Date	Temperature [a]	Humidity [a]	Strain	Age of larvae	Weighings	Total larvae weighed	Total weight	Average weight—	
									100 larvae	Each
		°F	*Percent*		*Hours*	*Number*	*Number*	*Milligrams*	*Milligrams*	*Milligrams*
A	May 21 to June 3, 1930.	73.0	60	Colorado	Up to 1	10	1,000	44.41	4.44	0.0444
				Virginia	do	19	1,900	82.80	4.35	.0435
				Virginia ♀ × Colorado ♂ F_1	do	10	1,000	45.05	4.50	.0450
				Colorado ♂ × Virginia ♀ F_1	do	10	1,000	43.41	4.34	.0434
B	July 21–28, 1930.	91.7	44	Colorado	do	18	1,800	70.68	3.92	.0392
				Virginia	do	17	1,700	63.22	3.71	.0371
				Virginia ♀ × Colorado ♂ F_1	do	5	500	18.27	3.65	.0365
				Colorado ♀ × Virginia ♂ F_1	do	5	500	19.61	3.92	.0392
C	June 2, 1932	80.8	63	Colorado	do	5	500	22.11	4.42	.0442
				Virginia	do	5	500	20.56	4.11	.0411
D	June 3–23, 1932.	74.7	100	Colorado	1½ to 2½	12	1,200	57.19	4.76	.0476
				Virginia	do	12	1,200	57.22	4.76	.0476
			72	Colorado	do	12	1,200	50.94	4.24	.0424
				Virginia	do	12	1,200	49.35	4.11	.0411

[a] Average at time of collecting and weighing.

In series D of table 8 is found the weight of newly hatched Colorado and Virginia larvae which were kept in a saturated atmosphere, compared with the weight of larvae kept at atmospheric humidity for the same length of time. There was no significant difference in the weight of the Colorado and Virginia larvae in the saturated atmosphere. The Colorado larvae under atmospheric conditions weighed slightly more than the Virginia larvae, and both strains weighed distinctly less than the larvae kept in a saturated atmosphere. The weight of the Colorado larvae under atmospheric conditions was 10.9 percent less and that of the Virginia larvae 13.7 percent less than the weight of the corresponding strain kept in a saturated atmosphere at the same time.

In this connection it should be stated that the weights of full-fed or mature larvae of the Colorado and Virginia strains were found to be nearly the same when weighed from September 11 to 16, 1929. In 10 weighings of 10 larvae each, the Colorado larvae slightly exceeded the Virginia larvae in 5 of the trials and were exceeded in weight by the Virginia larvae in the other 5 trials. The total weight of 100 full-fed Colorado larvae was 5,503.06 milligrams, as compared with 5,529.21 milligrams for 100 full-fed Virginia larvae. That is, the average weight of a full-fed Colorado larva was 55.03 milligrams and that of a Virginia larva 55.29.

ENDURANCE OF STARVATION

Newly hatched larvae were confined in Stender dishes for periods varying from 24 to 28 hours. The larvae were then examined and all individuals that showed signs of life when disturbed with a needle were included with the live larvae. Larvae of the strains to be starved were collected at the same time and confined in Stender dishes. For this work it was necessary to use a dish having a cover ground very accurately to fit, in order to prevent the escape of an occasional larva. Results of the tests are given in table 9. The Colorado larvae exhibited greater endurance to starvation than Virginia larvae in all 3 years in which comparisons were made. It is apparent that the number of larvae that survived increased as the temperature during the period of starvation was lowered. Little difference in endurance was shown between the Colorado and Colorado-K larvae in 1933. There was also little or no difference between Virginia and Virginia-K larvae, except in the tests during the cool weather of August 21, 1933, when the Virginia-K larvae survived in about the same proportion as the Colorado larvae.

TABLE 9.—*Endurance of starvation of young codling-moth larvae of the various strains*

Year	Date	Period of starvation	Temperature		Colorado strain			Virginia strain			Colorado-K strain			Virginia-K strain		
			Range	Mean	Larvae	Live larvae		Larvae	Live larvae		Larvae	Live larvae		Larvae	Live larvae	
		Hours	° *F*	° *F.*	*No.*	*No*	*Pct*	*No*	*No*	*Pct*	*No.*	*No*	*Pct*	*No*	*No*	*Pct*
1930	July 24	26 to 28	67–95	81	264	41	15.5	500	31	6.2						
1931	July 17	do	69–89	79	173	49		139	26							
	Aug 10	do	70–89	79½	279	44		309	45							
	Aug. 16	do	66–88	77	248	31		276	13							
		Total			700	124	17.7	724	84	11.6						
1933	Aug 6	24 to 26	59–83	71	302	105		447	108							
	Aug. 10	do	64–78	71	257	123		461	159		431	151		306	93	
	Aug. 12	do	64–90	77	287	49		398	62		108	23		181	37	
	Aug 18	do	67–87	77	98	20		150	28		144	28		129	28	
	Aug. 19	do	66–86	76				108	30		198	63		255	62	
		Total			944	297	31.4	1,564	387	24.7	881	265	30.0	871	220	25.2
	Aug. 21	24 to 26	62–73	67½	343	214	62.3	355	174	49.0	241	161	66.8	316	195	61.7

The average weight of the larvae that survived the starvation tests of July 30, 1930, was 0.024 milligram per larva for the Colorado strain and 0.019 milligram for the Virginia strain. A comparison of the average weight of a starved larva with the average weight of a recently hatched larva of the same strain as determined July 21 to 28, 1930 (table 8, series B), shows that during the period of starvation the average reduction in weight per larva was 0.015 milligram, or 38.4 percent for the Colorado strain, and 0.018 milligram or 48.6 percent for the Virginia strain. In other words, loss in weight was less for the Colorado larvae than for the Virginia larvae. This was also demonstrated in the comparison of weights made in series D (table 8). Loss in weight may result from hunger and loss in moisture. In the case of series D, the latter is probably the chief cause. Difference in the rate of loss of moisture may be due to a difference in water-binding colloids in the tissues of the larvae rather than to any difference in the structure of the epidermis.

FUMIGATION TESTS

EGGS

Comparative tests were made in which codling-moth eggs of various strains were fumigated with potassium cyanide. Two experiments in 1931 and three each in 1932 and 1933 were made, in which the eggs were fumigated for a period of 2 hours. During the season of 1933 there were eight fumigation tests, each for a period of 1½ hours. In each test in 1933 all eggs were fumigated at the same time in the same fumigation chamber; in the other years individual fumigation jars were used at the same time. The results are recorded in table 10. Eggs of three ages or stages of embryonic development were fumigated at the same time; in the first stage were eggs less than 24 hours old, mostly between 12 and 16 hours; in the second stage eggs intermediate between recently deposited eggs and eggs nearly ready to hatch; and in the third stage eggs due to hatch within 1 to 12 hours after they were placed in the fumigation chamber.

TABLE 10.—*Summary of experiments on the comparative tolerance of Colorado, Colorado–K, Virginia, and Virginia–K codling-moth eggs to fumigation with potassium cyanide*

Year	Duration of fumigation	Age of eggs	Colorado eggs			Virginia eggs			Colorado-K eggs			Virginia-K eggs		
			Total	Hatched		Total	Hatched		Total	Hatched		Total	Hatched	
	Hours	*Hours*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1933	1½	2–24	3,399	1,881	55.3	3,345	1,974	59.0	3,550	1,822	51.3	3,030	1,628	53.7
		60–84	2,191	1,068	48.7	1,801	559	31.0	2,085	1,157	55.4	1,752	627	35.7
		(a)	6,459	3,350	51.8	5,408	816	15.0	4,455	2,636	59.1	3,554	871	24.5
	2	2–24	1,337	602	45.0	1,344	793	59.0	1,774	731	41.2	1,709	794	46.4
		60–72	932	519	55.6	752	253	33.6	805	564	70.0	926	548	59.1
		(a)	1,571	179	11.3	1,734	4	.2	1,493	405	27.1	1,343	52	3.8
1932		2–24	310	161	51.9	1,001	519	51.8						
1931	2	2–24	606	350	57.7	1,031	487	47.2						
		(a)	596	341	57.2	1,472	77	5.2						

[a] Eggs mostly 5 to 6 days old, all over 100 hours old. The eggs were due to hatch within a few hours when fumigated

There was no indication of a significant difference in tolerance to fumigation between recently deposited eggs of the different strains. On the other hand, there was a marked difference in the tolerance of eggs almost ready to hatch. The order of tolerance was Colorado–K, Colorado, Virginia–K, and Virginia. This is the same order as that established by young larvae of the same strains in entering sprayed apples. Embryonic development was slowed down greatly in the fumigated eggs. Often an embryo developed into an apparently normal larva which perished without having sufficient strength to break through the chorion. In 1931 the temperature was an average of 7° lower at the time of the 2-hour fumigation tests than in 1933.

The difference in temperature and the fact that different fumigation chambers were used must be taken into consideration in comparing the results obtained in the experiments of the 2 years. In 1932 the temperature at the time of fumigation was about the same as the temperature in 1931.

Cotton (*2*) has shown that the susceptibility of an insect to a fumigant varies directly with the rate of respiratory metabolism. This explains why eggs almost ready to hatch were more susceptible to potassium-cyanide fumigation than recently deposited eggs, in which the respiratory rate is low. It will be observed, however, that the Colorado and Colorado-K eggs in the 1½ hours of fumigation in 1933 and the Colorado eggs in the 2 hours of fumigation in 1931 showed little or no decrease in the percentage of hatch of the eggs containing well-developed embryos as compared with the percentage of hatch for the youngest eggs fumigated at the same time. In these tests the majority of well-developed embryos in the Colorado and Colorado-K eggs were not carried beyond the critical stage for recovery, so that hatching occurred later; but the critical stage was passed in most of the eggs of the same strains and embryonic development in the 2 hours of fumigation in 1933. It is apparent that eggs of the different strains containing embryos in the late stages of development have different levels for the critical stage beyond which recovery is impossible. In other words, a difference in vigor or hardiness (that is, power of recovery) becomes apparent as the embryo develops and is most marked when the embryo is fully developed. The percentage of eggs having a high level of vigor or greater power of recovery differed with each strain. The greatest percentage was in the Colorado-K strain, a close second was the Colorado strain, next in order was Virginia-K, and lastly, Virginia.

YOUNG LARVAE

Newly hatched Colorado and Virginia larvae were fumigated with potassium cyanide and then placed on whole apples or on slices of apples and kept in Stender dishes for 24 hours. A count was then made of the dead and live larvae. The results are given in table 11.

TABLE 11.—*Summary of experiments on the comparative tolerance of newly hatched Colorado and Virginia codling-moth larvae to fumigation with potassium cyanide*

Year	Experiments	Fumigation period	Food provided larvae after fumigation	Colorado strain			Virginia strain		
				Larvae fumigated	Larvae alive 24 hours after fumigation		Larvae fumigated	Larvae alive 24 hours after fumigation	
	Number	*Hours*		*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1931	9	1	Whole apples	444	142	31.98	534	78	14.6
	3	1	Sliced apples	141	92	65.24	135	39	28.8
	16	2	Whole apples	753	148	19.65	908	50	5.5
	9	2	Sliced apples	393	162	41.22	428	90	21.0
1932	13	2	do	1,847	615	33.29	2,424	396	16.3
1933	21	2	do	2,101	639	30.41	2,172	355	16.3
1931	4	(a)	Whole apples	183	132	72.13	219	118	53.8
	5	(a)	Sliced apples	209	185	88.51	211	176	83.4

[a] Not fumigated.

It is apparent that the Virginia larvae were more susceptible to potassium-cyanide fumigation than the Colorado larvae. There was a higher percentage of live larvae of each strain when the fumigated larvae were placed on sliced apples after fumigation than when placed on whole apples. Many of the larvae on whole apples did not recover sufficiently to enter the fruit and died from starvation. In short, the power of recovery was possessed to a greater degree by more of the Colorado larvae than the Virginia larvae. It is reasonable to assume that the power of recovery is an index of the vigor inherent in the larvae. A similar but less marked difference was demonstrated by the check experiments.

LARVAE OF SECOND AND LAST INSTARS

Colorado and Virginia larvae of the second instar (7 days old) were removed from apples and fumigated with potassium cyanide. The fumigated larvae were then placed on slices of apples in Stender dishes and examined 24 hours later. The results are shown in table 12. A higher percentage of Colorado larvae survived the fumigation tests. The check experiments also showed a higher percentage of Colorado larvae able to survive being transferred from their natural environment in the apple to slices of apples in Stender dishes.

TABLE 12.—*Summary of experiments on the comparative tolerance of second-instar and also of mature or full-fed larvae of the Colorado and Virginia codling-moth strains to fumigation with potassium cyanide, 1933*

Larval stage	Experiments	Fumigation period	Colorado strain			Virginia strain		
			Larvae fumigated	Larvae alive 24 hours after fumigation [a]		Larvae fumigated	Larvae alive 24 hours after fumigation [a]	
	Number	*Minutes*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Second instar	3	45	77	54	70.1	77	45	58.4
	1	60	24	16	66.6	25	10	40.0
	8	75	193	72	37.3	208	46	22.1
	4	([b])	92	82	89.1	93	70	75.2
Last instar	12	300-420	1,516	857	56.53	1,537	713	46.38

[a] Second-instar larvae were placed on sliced apples after fumigation and kept in Stender dishes

[b] Not fumigated.

Full-fed larvae of the two strains were also fumigated with potassium cyanide and then kept in museum jars for 24 hours before they were examined. All larvae showing a trace of life when disturbed with a needle were considered alive. In all tests the larvae were fumigated in the same jar at the same time. The results (table 12) show a slight difference in survival in favor of the Colorado larvae. The difference does not appear marked as shown by larvae of the first instar or by the eggs containing embroyos in the late stage of development.

SUMMARY AND CONCLUSIONS

The history of the codling moth in the Grand Valley of Colorado and the Shenandoah Valley of Virginia shows that the insect has been much more difficult to control in Colorado. The seasonal history of the codling moth is essentially alike in the two districts, according to published records.

Larvae from Colorado and native Virginia larvae have been reared and studied under the same climatic environment in Virginia for 7 years. This paper reports results of the investigations of the past 5 years.

Colorado larvae reared under Virginia climatic conditions since 1928 have consistently demonstrated a distinct superiority over Virginia larvae in their ability to enter sprayed fruit.

Greater ability of the Colorado larvae to enter sprayed fruit was not specific for lead arsenate but was also demonstrated when such nonarsenical sprays were used as cryolite, barium fluosilicate, rotenone, cuprous cyanide, and nicotine.

From 1929 to 1933 larvae of Colorado origin and native Virginia larvae were reared on fruit freshly sprayed with lead arsenate. These larvae were designated as Colorado-K and Virginia K strains, respectively, to distinguish them from the Colorado and Virginia larvae reared on unsprayed apples or on fruit carrying a very small amount of lead-arsenate residue.

In 1933 the Colorado-K, Colorado, Virginia-K, and Virginia larvae demonstrated their comparative ability to enter sprayed fruit in the order named, that is, the Colorado-K larvae were most successful and the Virginia larvae least successful in all tests regardless of the kind of insecticide used. Comparative tests were made on fruit sprayed with cryolite, cuprous cyanide, nicotine, and lead arsenate.

Colorado-Virginia crosses, through 10 generations, maintained an intermediate position between the parent strains with respect to their ability to enter fruit sprayed with lead arsenate.

Back crosses between the F_5 generation of the cross Virginia female × Colorado male and Colorado moths demonstrated a marked increase in ability to enter sprayed fruit, while the back cross with Virginia codling moths showed a tendency to approach the Virginia strain in ability to enter the sprayed fruit.

Colorado larvae of the second instar were slightly superior to Virginia larvae in ability to enter unsprayed and sprayed fruit.

Larvae of Colorado and Virginia strains were more successful in entering unsprayed fruit as well as sprayed fruit when the temperature was relatively high than in cooler weather.

Larvae of the Colorado strain under all conditions were consistently more successful than Virginia larvae in entering unsprayed fruit.

Careful observations on the habit of rejecting apple tissue while entering the fruit failed to reveal any difference in habit of entering that could be used to explain the difference in survival of the Colorado and Virginia larvae under observation. By actual count the number of rejections made by the Colorado larvae did not exceed those made by the Virginia larvae.

At atmospheric temperature and humidity, the average weight of recently hatched Colorado larvae was slightly greater than that of Virginia larvae. Recently hatched larvae in a saturated atmosphere weighed the same for both strains. Under atmospheric conditions the average weight of recently hatched larvae of the Colorado-Virginia crosses varied from slightly less than the average weight of Virginia larvae to slightly more than the average weight of Colorado larvae.

Colorado larvae endured starvation more successfully than Virginia larvae. The percentage of survival increased for both strains when the larvae were starved during cool weather in comparison with survival when the temperature was relatively high.

Loss in weight of starved larvae was greater for Virginia larvae than for Colorado larvae. Difference in the weights of Colorado and Virginia larvae under atmospheric conditions may be due to loss of moisture. The rate of loss of weight between the strains may be due, theoretically, to a difference in water-binding colloids in the tissues of the larvae rather than to any structural difference in the epidermis.

There was little or no difference in tolerance to potassium cyanide fumigation between recently deposited eggs (less than 24 hours old) of the four strains.

There was a distinct difference in tolerance to potassium cyanide fumigation of eggs of the four strains when the eggs contained embryos in the late stages of development. The percentage of hatch was greatest for Colorado-K, then Colorado, next Virginia-K, and lastly Virginia eggs.

There was a progressive increase in susceptibility to the fumigant as the embryo developed in the eggs of all strains but the increase in susceptibility was greatest for Virginia eggs and least for Colorado-K eggs.

Newly hatched larvae of the four strains showed the same order of tolerance as did the eggs with well-developed embryos when subjected to potassium cyanide fumigation.

The essential difference in the strains was demonstrated in the partially developed embryo, was most marked in the fully developed embryo and the newly hatched larvae, and disappeared to a large extent in the full-fed larvae. The difference seems to be one of general vigor, or power of recovery, inherent in the individual.

This investigation demonstrates the existence of different strains of the codling moth in which the young larvae vary greatly in vigor. By rearing the larvae continuously on freshly sprayed fruit in the laboratory, it was possible to increase in a strain the proportion of individuals which possessed more vigor, with the result that a greater percentage of the young larvae entered and injured sprayed apples. Difference in vigor appeared in the well-developed embryo and was most marked in the fully developed embryo and in newly hatched larvae. It was also quite evident in larvae of the second instar but had disappeared to a large extent in the full-fed or mature larvae.

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THE NUTRITIVE VALUE OF THE PROTEINS OF ALFALFA HAY AND CLOVER HAY WHEN FED ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN[1]

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INTRODUCTION

The great importance of legume hay is recognized by everyone interested in livestock production. Because of this importance, it is highly desirable to obtain data on the nutritive value of the proteins in these hays when they are fed alone and when fed in combination with the protein of corn and other farm-grown grains.

It is apparent that the great value of legume hays in stock feeding is largely due to their heavy yield per acre, their high protein content, and their palatability. From the good results which are obtained with legume hay, even when animals are given little or no other feeds in addition, as is sometimes the case in the alfalfa districts of the West, it might also be assumed that the protein of legume hay has a high quality or high biological value. The results of experiments thus far reported, however, do not seem to bear out this assumption. Practically all experiments in which alfalfa hay furnished the principal or sole source of protein have indicated that the crude protein possesses a low nutritive value. Sotola (*20*)[2] found the protein of alfalfa hay to have a biological value of only 56 when fed alone to lambs. This was considerably lower than values for corn silage or even for sunflower silage. A later report by Sotola (*21*) indicated still lower biological values for alfalfa protein when fed to lambs. This report gave the protein of the alfalfa stems a value of 64, while for the protein in the leaves it was only 44 and for the protein in the whole hay it was 51.

Haag (*3*) made a study of the factors limiting the nutritive value of the crude protein of alfalfa for rats, and reported a deficiency of cystine, one of the essential amino acids, in the proteins of this hay. Nevens (*17*) fed alfalfa to rats at a 10 percent level of intake and obtained an average biological value of 62. His results further indicated no supplementary effect of the corn-grain proteins with alfalfa proteins, since the combination gave biological values averaging 58.

Very few data reported show any marked differences in efficiency of protein utilization when common feeding stuffs have been fed to ruminants. Experiments by Hart, Humphrey, and Morrison (*7*) to determine the nutritive value of nonprotein nitrogen for dairy heifers indicated that the total nitrogen of alfalfa hay possesses approximately the same efficiency for growth and milk production as the nitrogen of corn grain plus corn stover. Hart and Humphrey (*4*) showed that milk protein is more efficient than corn or wheat protein when fed with corn stover. These same investigators (*5*) also found that both

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[2] Reference is made by number (italic) to Literature Cited, p. 569.

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linseed meal and dried distillers' grains provided a more efficient combination of proteins with corn stover than did corn gluten feed, but in later work (*6*) no differences were found among the three concentrates when clover hay was used as the roughage. Experimental results obtained by Maynard, Miller, and Krauss (*9*) indicated that a ration for milk production based on clover hay furnishes a combination of proteins no more efficient than one based on timothy hay. Morris and Wright (*16*) reported experiments from which they concluded that the nitrogen of beans was superior to that of meat meal or linseed meal for milk production. Each of the feeds in question was fed with a ration of straw, beet pulp, and oats.

In view of the results indicating a rather low quality of protein in alfalfa hay it seemed worth while to obtain further data on this subject and to compare the nutritive value of alfalfa protein with that furnished by clover hay. Since legume hays are rarely fed alone, but are commonly fed in combinations with the cereals or other farm-grown feeds, it would seem that protein studies on these combinations would also be of considerable practical value.

In the series of experiments here reported, nitrogen metabolism studies were conducted with five growing wether lambs. It is believed that the results are, to a certain extent, applicable to cattle as well as to sheep. In these studies alfalfa hay and clover hay have each been fed as the only source of protein and each of these hays has also been fed with corn grain. For each ration the digestibility of the protein, the percentage of protein stored, and the biological value of the protein have been determined and are herein reported.

EXPERIMENTAL METHODS

Five young growing wether lambs have been used in three nitrogen balance experiments. Two lambs were used in the first experiment. They were crossbreds of fairly good type and were thrifty. Three purebred lambs of excellent type were used in the second and third experiments. The lambs were kept in metabolism crates similar in construction to those described by Forbes (*2*). The experimental collection periods were 10 days in length in all the experiments. The intervals between collection periods were also 10 days, which provided ample time to adjust the food intake to a uniform amount that would be consumed daily. Excreta collections were not started until each lamb had been eating the same amount of the ration for at least 6 days.

The feed for each experimental period was weighed out at one time, the daily feed for each lamb being placed in individual paper bags. A representative sample was taken at this time for chemical analysis and served as the basis for computing the nutrients fed. The daily ration for each lamb was fed in 2 equal portions during the first experiment, but the lambs were fed 3 times daily during the second and third experiments. Any unconsumed feed was collected, air-dried, finely ground, and sampled for analysis.

Quantitative collections of urine were made each day, and the urine, together with the washings from the crates, was preserved with toluene in tightly stoppered carboys in a refrigerator room at a temperature of approximately 5° to 10° C. Aliquots from the total volume for each collection period were taken for analysis after the completion of each period.

The daily collections of feces were preserved in airtight containers with acidified alcohol and were stored in the refrigerator. The total quantity for each collection period was then dried at a temperature not exceeding 60° C., weighed, finely ground, and carefully sampled for analysis.

The methods of analysis used were those of the Association of Official Agricultural Chemists (*1*), with the exception of the nitrogen determinations. Nitrogen was determined by the boric acid modification of the Kjeldahl method (*18*).

Several methods may be used in comparing the efficiency of proteins. The writers have used the ordinary coefficient of apparent digestibility, the storage of protein as calculated by the method of McCollum (*8*), and the biological value as computed by the Mitchell method (*10*). The last-named method presumably gives the biological value of a protein or mixture of proteins for both growth and maintenance.

FIRST EXPERIMENT

Two wether lambs were used in this experiment to determine the digestibility, storage of protein, and biological value of the proteins of alfalfa hay, clover hay, and combinations of each with corn protein.

The percentage composition of feeds used is shown in table 1. All the feeds were of excellent quality. The alfalfa hay was second cutting and rather fine-stemmed and leafy. All hay was chopped into lengths ranging from one half to three fourths of an inch. A choice grade of yellow ground corn was used. The composition of each ration as fed is shown in table 2.

TABLE 1.—*Percentage composition of feeds used in the first experiment*

Feed	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Alfalfa hay	93.01	6.57	15.83	2.02	29.41	39.18
Clover hay	90.67	5.59	12.72	2.89	29.03	40.44
Corn, yellow	90.62	1.60	9.15	4.60	1.79	73.48

TABLE 2. *Percentage composition of experimental rations in the first experiment*

Constituent	Low-nitrogen ration	Alfalfa-hay ration	Clover-hay ration	Alfalfa-hay and corn ration	Clover-hay and corn ration
Straw	40.0				
Alfalfa hay		63.5		47.4	
Clover hay			78.7		59.0
Corn, yellow				27.3	27.3
Cornstarch	23.5	14.6	11.2	6.4	3.4
Cane sugar	23.5	12.5	7.1	5.4	2.3
Cellulose, regenerated	5.0	4.5		8.0	5.0
Corn oil	4.5	2.9	1.0	3.5	1.0
Minerals [a]	3.5	2.0	2.0	2.0	2.0
Total	100	100	100	100	100
Protein content (N×6.25) [b]	1.92	10.09	10.11	10.46	10.35

[a] The mineral mixture contained equal parts of steamed bone meal, ground limestone, and sodium chloride.

[b] These percentages of nitrogen are the averages of analyses of 2 mixes of each ration used.

An attempt was made to feed the lambs a purified diet practically free of nitrogen, but this was unsuccessful and it was necessary to use a low-nitrogen ration containing some wheat straw in addition to the purified ingredients. The nitrogen in the straw was probably not very digestible and this would result in an over-estimation of the metabolic nitrogen. Since a ration entirely free of nitrogen could not be used, however, it was believed that this ration would be fairly satisfactory. Morgen and his coworkers (*15*) used a low-nitrogen ration for sheep, consisting of straw (treated to remove most of the nitrogen), starch, sugar, oil, and minerals. They found that the average excretion of metabolic nitrogen in the feces was 0.51 g for each 100 g of dry matter consumed. Sotola (*20*) fed a similar low-nitrogen ration to sheep and found the average excretion of metabolic nitrogen in the feces to be 0.65 g for each 100 g of dry matter fed. The data obtained in the three experiments reported in the present paper show an average excretion of 0.56 g of metabolic nitrogen for each 100 g of dry matter consumed. It is apparent, therefore, that this value agrees quite closely with those obtained by Morgen and his coworkers (*15*) and Sotola (*20*).

The experimental rations consisted of this low-nitrogen ration and four other rations comparable in character but with the straw and part of the starch and sugar replaced by enough of each feed to furnish approximately 10 percent of protein. In the two rations containing corn, 25 percent of the total protein was supplied by the corn and 75 percent by the hay. All the rations were equalized, as nearly as possible, in regard to dry matter, crude fiber, and total energy content. The rations were all adequate in mineral content. Sufficient vitamins were undoubtedly furnished by all rations, except the low-nitrogen ration. It was believed, however, that this would not be a limiting factor in short balance experiments of this type. The addition of vitamin B supplements would have added nitrogen to the low-nitrogen ration, the value of which would not be known. Regenerated cellulose[3] was used in various amounts to help equalize the "roughage" content of the rations. The lambs were rotated on the different rations according to the customary procedure.

The low-nitrogen ration was fed during the initial and final periods of the experiment. The collection periods were not started until the nitrogen excretion in the urine had apparently reached a constant level as indicated by analyses of the urine for a number of consecutive days. From these collections the losses of nitrogen in the feces and urine were determined and used in estimating, during the periods of protein feeding, the amounts of food nitrogen present in the urine and feces. These data were used in calculating the body's contribution to the feces, or the so-called "metabolic nitrogen", which was expressed in grams of nitrogen per 100 g of dry matter in food eaten. The contribution of the body to the urine was computed in grams of nitrogen per kilogram of body weight. These values were assumed to change in a linear fashion, with respect to time, in the intervening periods between the initial and final low-nitrogen periods.

The results of the first metabolism experiment with the significant intermediate computations are presented in table 3.

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[3] Obtained as washed Sylphrap.

TABLE 3.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the first experiment* [a]

Wether no.	Body weight: Initial	Body weight: Final	Body weight: Average	Food intake	Dry matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
1	18.18	17.21	17.70	2,066	1,987.3	6.19	10.68	[b]0.537	---	---	7.64	[c]0.432	---	---	---	---	---	---
2	25.14	24.75	24.95	4,000	3,853.6	11.52	18.48	[b].479	---	---	11.98	[c].480	---	---	---	---	---	---
CLOVER-HAY RATION																		
1	20.36	21.07	20.72	6,000	5,658.0	98.46	43.07	32.25	10.82	87.64	16.98	9.95	7.03	80.61	56	39	69	92
2	27.83	28.14	27.99	8,000	7,523.2	127.52	64.55	33.85	30.70	96.82	33.26	13.44	19.82	77.00	49	23	47	80
ALFALFA-HAY RATION																		
1	21.88	22.79	22.34	6,000	5,683.2	95.52	34.67	33.82	0.85	94.67	26.18	11.84	14.34	80.33	64	36	57	85
2	25.70	26.93	26.32	8,000	7,574.1	130.96	61.29	35.22	26.07	104.89	32.37	12.63	19.74	85.15	53	28	54	81
CLOVER-HAY AND CORN RATION																		
1	25.32	25.87	25.60	6,000	5,551.2	100.62	43.36	36.08	7.28	93.34	28.88	16.13	12.75	80.59	57	28	50	86
2	29.20	29.83	29.52	8,000	7,383.2	130.64	58.46	31.75	26.71	103.93	33.70	14.17	19.53	84.40	55	29	53	81
ALFALFA-HAY AND CORN RATION																		
1	24.02	24.90	24.46	6,000	5,596.8	98.76	30.91	34.98	[d]0	98.76	26.58	14.19	12.39	86.37	69	42	61	87
2	30.92	31.99	31.46	8,000	7,440.8	135.92	48.38	30.88	17.50	118.42	41.31	15.10	26.21	92.21	64	34	53	78
LOW-NITROGEN RATION																		
1	23.91	21.96	22.94	1,990	1,886.9	7.46	12.77	[b]0.677	---	---	15.60	0.680	---	---	---	---	---	---
2	28.90	28.13	28.52	3,566	3,389.1	10.66	13.71	[b].405	---	---	13.68	[c].480	---	---	---	---	---	---

[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

[d] Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no food nitrogen was present in the feces.

The data seem to show that lamb no. 1 was more efficient in utilizing protein than the second lamb. However, greater difficulty was encountered in getting the first lamb to eat the low-nitrogen ration, and it lost considerable weight before it would consume enough food to maintain its body weight. It is possible that when protein feeding was resumed, considerable amounts of the food nitrogen were used for repair and formation of tissue, which resulted in a larger percentage of nitrogen being stored than if the animal had been in a more nearly normal condition. However, Mitchell (*13*) believes that a period of low-nitrogen feeding will exert no appreciable effects upon the utilization of protein in subsequent experimental periods. It would seem, therefore, that the first lamb was simply more efficient in utilizing protein.

Some individual variations can be noted in the apparent digestibility of the protein, the alfalfa being somewhat higher than the clover. The combination of alfalfa and corn had the highest apparent digestibility of the four rations used. The largest storage of protein was obtained with the alfalfa and corn ration; the results with the other three rations were approximately the same. No consistent differences can be noted in the storage of digestible nitrogen.

In regard to the biological values, it is quite evident that no difference in utilization of the absorbed nitrogen has been measured with the different rations. However, the values are quite high and indicate a much greater percentage utilization of nitrogen than has been reported by other investigators for these feeds, especially for alfalfa hay.

SECOND EXPERIMENT

Since no definite conclusions could be drawn from the limited data furnished by the first experiment, the work was repeated with three lambs. The feeds were of approximately the same quality and chemical composition (table 4) as those used in the first experiment, and the same general plan was followed in making up rations and conducting the experiment. This trial, therefore, was simply a duplication of the first one.

TABLE 4.—*Percentage composition of feeds used in the second experiment*

Feed	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Alfalfa hay	93.62	6.38	16.94	2.22	29.28	38.80
Clover hay	94.21	4.99	12.25	2.07	30.41	44.49
Corn, yellow	91.83	1.20	9.82	4.08	2.03	74.70

The results of the metabolism trials are shown in table 5, together with the apparent digestibility, nitrogen storage, and the final biological values of the proteins. These data are in agreement in showing a higher apparent digestibility for the alfalfa protein than for the clover protein. The combination of proteins furnished by clover and corn was more digestible than that of clover hay alone; the alfalfa and corn combination of proteins had the highest apparent digestibility of the four rations fed. When allowance is made for the metabolic fecal nitrogen and the true digestibility of the food nitrogen calculated, the same relative differences in digestibility are still shown. The composition of the rations as they were fed is given in table 6.

TABLE 5.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the second experiment* [a]

Wether no.	Body weight			Food intake	Dry-matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine[c]	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
3	23.20	21.63	22.42	2,650	2,567.62	6.12	13.54	[b] 0.527			7.58	[c] 0.338						
4	23.53	21.60	22.57	2,326	2,250.76	4.61	12.73	[b] .566			15.53	[c] .688						
5	28.23	26.67	27.45	3,449	3,336.67	8.08	14.80	[b] .444			12.40	[c] .452						
CLOVER-HAY RATION																		
3	24.47	25.00	24.74	8,000	7,656.00	128.56	64.30	43.18	21.12	107.44	34.66	8.58	26.08	81.36	50	23	46	76
4	24.50	25.30	24.90	8,000	7,656.00	128.56	68.45	46.01	22.44	106.12	33.49	15.79	17.70	88.42	47	21	44	83
5	31.17	32.47	31.82	10,000	9,469.00	164.50	84.01	43.18	40.83	123.67	45.23	12.89	32.34	91.33	49	21	44	74
ALFALFA-HAY RATION																		
3	25.27	26.33	25.80	8,000	7,670.40	124.64	55.46	46.10	9.36	115.28	37.89	9.18	28.71	86.57	56	25	45	75
4	25.43	26.73	26.08	8,000	7,670.40	124.64	54.75	48.78	5.97	118.67	38.54	15.10	23.44	95.23	56	25	45	80
5	28.80	30.37	29.59	9,383	9,069.61	142.34	69.28	40.81	28.47	113.87	40.04	12.69	27.35	86.52	51	23	45	76
CLOVER-HAY AND CORN RATION																		
3	30.63	31.17	30.90	8,000	7,468.00	130.08	59.25	50.41	8.84	121.24	28.54	11.53	17.01	104.23	54	33	60	86
4	28.57	29.60	29.09	8,000	7,468.00	130.08	62.26	52.72	9.54	120.54	42.48	13.67	28.81	91.73	52	19	37	76
5	32.57	33.93	33.25	10,000	9,452.00	159.90	67.84	43.67	24.17	135.73	50.30	12.67	37.63	98.10	58	26	45	72

[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The changes in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in case of metabolic nitrogen in the feces.

TABLE 5.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the second experiment*—Continued

ALFALFA-HAY AND CORN RATION

Wether no.	Body weight			Food intake	Dry matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
3	27.83	28.90	28.37	8,000	7,652.80	120.96	45.51	48.82	[d] 0	120.96	38.71	10.36	28.35	92.61	62	30	49	77
4	26.97	28.00	27.49	8,000	7,652.80	120.96	49.37	51.35	[d] 0	120.96	40.53	14.43	26.10	94.86	59	26	43	78
5	34.47	36.50	35.49	10,000	9,495.00	154.00	58.56	44.34	14.22	139.78	61.47	12.67	48.80	90.98	62	22	36	65
LOW-NITROGEN RATION																		
3	29.07	27.70	28.39	2,810	2,697.86	7.66	19.21	[b] 0.712			10.85	[c] 0.382						
4	29.23	27.70	28.47	3,452	3,315.63	9.58	24.57	[b] .741			11.81	[c] .415						
5	36.30	35.13	35.72	4,119	3,952.66	11.62	18.66	[b] .472			11.91	[c] .333						

[b] Fecal nitrogen per 100 g. of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in case of metabolic nitrogen in the feces

[d] Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no food nitrogen was present in the feces.

TABLE 6.—*Percentage composition of experimental rations in the second experiment*

Constituent	Low-nitrogen ration	Alfalfa-hay ration	Clover-hay ration	Alfalfa-hay and corn ration	Clover-hay and corn ration
Straw	40.0				
Alfalfa hay		59.1		44.3	
Clover hay			81.7		61.2
Corn, yellow				25.5	25.5
Cornstarch	23.5	15.5	7.7	7.6	2.7
Cane sugar	23.5	15.4	7.6	7.6	2.6
Cellulose, regenerated	5.0	5.0		9.0	4.0
Corn oil	4.5	3.0	1.0	4.0	2.0
Minerals [a]	3.5	2.0	2.0	2.0	2.0
Total	100	100	100	100	100
Protein content (N×6.25) [b]	1.63	9.61	10.16	9.54	10.08

[a] The mineral mixture contained equal parts of steamed bone meal, ground limestone, and sodium chloride.

[b] These percentages of nitrogen are the averages of analyses of 2 mixes of each ration used.

All the lambs showed approximately the same efficiency in storing protein from the alfalfa and clover rations. Some variation was noted in the percentage of protein storage by the individual animals on the other two rations, but the average values are approximately the same. The biological values are again high, as they average 78 for clover protein, 77 for alfalfa protein, 78 for the clover and corn combination, and 73 for the alfalfa and corn combination. The lower average value for the mixture of alfalfa and corn is due chiefly to the one lower value given by lamb no. 5. The results thus agree quite closely with those of the first experiment, and for convenience of study the results of both experiments have been summarized together for discussion.

DISCUSSION OF FIRST AND SECOND EXPERIMENTS

A summary of the first and second experiments is presented in table 7.

TABLE 7.—*The digestibility and biological value of clover and alfalfa proteins when fed to lambs alone and in combination with corn protein*

Item	Percentage digestibility and biological value when fed to lamb no.					Average
	1	2	3	4	5	
Clover protein:						
Apparent digestibility	56	49	50	47	49	50
Percentage total nitrogen stored	39	23	23	21	21	25
Percentage digestible nitrogen stored	69	47	46	44	44	50
Biological value	92	80	76	83	74	81
Alfalfa protein:						
Apparent digestibility	64	53	56	56	51	56
Percentage total nitrogen stored	36	28	25	25	23	27
Percentage digestible nitrogen stored	57	54	45	45	45	49
Biological value	85	81	75	80	76	79
Clover and corn protein:						
Apparent digestibility	57	55	54	52	58	55
Percentage total nitrogen stored	28	29	33	19	26	27
Percentage digestible nitrogen stored	50	53	60	37	45	49
Biological value	86	81	86	76	72	80
Alfalfa and corn protein:						
Apparent digestibility	69	64	62	59	62	63
Percentage total nitrogen stored	42	34	30	26	22	31
Percentage digestible nitrogen stored	61	53	49	43	36	48
Biological value	87	78	77	78	65	77

Averaging together the values obtained with the five animals used during the 2 years, it is found that the average coefficients of apparent digestibility are 56 percent for alfalfa protein, 50 percent for clover protein, 63 percent for the protein in the combination of alfalfa and corn, and 55 percent for the protein in the combination of clover and corn. The application of Student's method for significance of means shows that there is a significant difference in the digestibility of the protein in alfalfa hay and in clover hay. The odds are 188 to 1 in favor of alfalfa protein. Statistical treatment also shows a significant difference between the digestibility of the protein of each hay alone and of the hay fed in combination with corn, the odds in every case being greater than 100 to 1. These same significant differences are shown by the data for true digestibility of the food protein. The alfalfa protein was 89 percent digestible when allowance is made for the metabolic nitrogen in the feces, while the protein of clover hay was 81 percent digestible. The mixture of alfalfa and corn proteins gave a true digestion coefficient of 96 and the clover and corn mixture a coefficient of 89.

The nitrogen intake for each lamb on the different comparable rations was kept at approximately the same level, and it would seem tha nitrogen storage could be used with some reliability as a measure of protein efficiency. The average percentages of total nitrogen stored were 27 percent for alfalfa hay, 25 percent for clover hay, 31 percent for alfalfa hay and corn, and 27 percent for clover hay and corn. The differences between these average values are not very marked and statistical treatment indicates possible significant differences with only two comparisons. Greater storage was obtained from the alfalfa and corn ration than from the alfalfa ration. The odds are 23 to 1 against this difference occurring as a result of chance alone. These odds would indicate a tendency toward significance. A possible significant difference was also measured in protein storage between the alfalfa and corn ration and the clover ration. The odds in this case are 49 to 1 against this difference being due to chance.

The data further indicate no significant differences between the rations when the percentages of digestible nitrogen stored are considered. Some individual variations can be noted between experimental animals, but the final averages are practically identical for the four rations.

In these experiments it was intended to have the protein intake sufficiently high to produce some growth of the animals. This was accomplished as indicated by the live weights of the lambs and by the protein storage which was obtained.

A study of the individual biological values shows some variations, which can probably be attributed chiefly to the individual utilization of protein by each lamb. It is believed, however, that all the values are within the range of animal variation in experiments of this type. The final averages of the biological values show no significant difference between alfalfa protein and clover protein, these values being 79 for alfalfa and 81 for clover. This small difference is due largely to the one high value given by lamb no. 1 for clover protein. Furthermore, the combination of alfalfa hay and corn or clover hay and corn was no more efficient, as measured by the biological value, than these legume hays fed as the only source of protein. The average value for the alfalfa and corn combination was 77, and for the clover and corn combination it was 80.

It is quite obvious that no consistent differences in efficiency of protein utilization have been measured. These biological values for alfalfa hay are much higher than those reported by Sotola (*20, 21*).

Moreover they are considerably higher than those found by Nevens (*17*) in experiments with rates. However, they are in fair agreement with those for alfalfa hay fed to dairy heifers by Hart, Humphrey, and Morrison (*7*) as reported by Mitchell (*14*).

The question naturally arising, after the high biological values were obtained in these experiments, is why are they consistently higher than those reported by most other investigators for alfalfa hay? Since only very few experiments with ruminants have ever shown any measurable differences in efficiency of protein utilization, there is the possibility that the ruminant has the ability to synthesize certain of the essential amino acids as a result of bacterial action in the digestive tract. This possibility has been pointed out at various times in the literature.

Even if sheep do have the ability to synthesize some of the essential amino acids and hence have given high biological values for protein, this does not explain why the values obtained in these experiments are so much higher than those reported by Sotola (*20*, *21*). It is probable that the difference in concentration of protein in the rations would tend to produce a difference in the biological values. Mitchell (*11*) has shown for several proteins that an increase in the level of protein intake results in a lowering of the biological value of the protein. Sotola's (*20*) lambs were fed at a level of approximately 14.2 percent protein, on a dry basis, whereas in the writers' experiments the concentration of protein in the rations was at a level of 10.4 percent. The question remains as to whether the difference in plane of protein intake is entirely responsible for the wide differences in biological value.

There was also a decided difference in the net energy or total digestible nutrient content of the alfalfa-hay ration fed in these investigations and the alfalfa hay as fed by Sotola. The alfalfa ration used in the writer's experiments contained approximately 68 percent of total digestible nutrients, whereas alfalfa hay alone furnishes about 50 percent. It seems quite possible that this difference in net energy and total digestible nutrients may have been a factor in causing the difference in biological values. If fed a ration which is rich in protein and low in net energy, an animal may be unable to use part of the protein for growth, and will of necessity use part of it as a source of energy, thus wasting the nitrogen contained in this portion and lowering the biological value.

THIRD EXPERIMENT

A third experiment was conducted to determine whether low biological values, corresponding to those secured by Sotola, would be obtained if alfalfa hay was given to these lambs as the only feed, as was done in his experiments, and then to find whether high values, such as were previously obtained in the present studies, would be secured if the lambs were changed to an alfalfa ration which supplied plenty of net energy and furnished a lower plane of protein intake. The three wethers used in the second experiment were again employed. The last low-nitrogen period of the previous trial was taken as the first period of this experiment. The lambs were then placed on a ration of alfalfa hay alone with no supplements except common salt. After the period on alfalfa alone, the lambs were fed a ration in which the alfalfa hay was supplemented with starch, sugar, cellulose, corn oil, and minerals. This ration was just the same as the alfalfa rations fed in the first and second experiments, except that a different sample of alfalfa was used. The protein content of the ration was approximately 10 percent, but the dry-matter and crude-fiber content was about the same as that of alfalfa when fed alone. The lambs were finally placed on the low-nitrogen ration, which had the same composition as those previously fed.

The alfalfa hay used in this trial had the following chemical composition: 8.36 percent moisture, 7.30 percent ash, 17.01 percent protein, 29.86 percent crude fiber, 1.93 percent fat, and 35.54 percent nitrogen-free extract.

The results of the metabolism trials, with the intermediate calculations and final computations, are shown in table 8. Considerable differences were found in the apparent digestibility of alfalfa protein with these two methods of feeding. The protein of alfalfa fed alone had an apparent digestibility of 71 percent, while with the supplemented alfalfa ration the apparent digestibility of the protein was 56 percent. That starch and other nonnitrogenous material may have a depressing effect upon digestibility has been suggested by many writers. However, Mitchell (*12*) has pointed out that the differences in apparent digestibility observed may be explained entirely on the basis of the demonstrated ability of carbonaceous feed to cause an excretion of metabolic nitrogen in the feces in proportion to its dry-matter content. When the values for apparent digestibility in this experiment were corrected for the metabolic nitrogen in the feces there was no difference in the true digestibility. The average true digestibility of alfalfa alone was 92.5 percent, while the supplemented alfalfa ration had a true digestibility of 92.0 percent.

The biological values of the alfalfa proteins obtained with these two rations are particularly interesting. The average biological value of alfalfa protein fed alone was only 50 as compared with 77 for alfalfa fed with starch and sugar in the second experiment with the same lambs. When the lambs were put back on the ration of alfalfa plus starch and sugar, the values averaged 72, agreeing fairly well with those previously obtained with this ration. These data indicate that the low biological values obtained for alfalfa protein when alfalfa hay is given to sheep as the only feed are probably due to the particular method of experimentation and not to any deficiency of the protein.

Biological values of proteins are useful in comparing proteins only under definitely standardized conditions and systems of feeding. In order to secure biological values for the proteins in various feeds that will represent the actual relative nutritive values, it is necessary that the experimental rations furnish the same plane of protein intake. Moreover, they must provide a sufficient supply of digestible nonnitrogenous nutrients, so that there will be no wastage of proteins in the rations due to an excessive amount or to an insufficient supply of net energy in other nutrients. If digestible protein is provided in excess of the animal's requirements, or if insufficient net energy is furnished by the nonnitrogenous nutrients to permit full utilization of the protein in the ration, some of it will be deaminized and the nitrogen wasted. Consequently the biological value of the protein will be lowered, not as a result of any deficiency in the quality of the protein, but because of the method of experimentation.

TABLE 8.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the third experiment* [a]

Wether no.	Body weight, Initial	Body weight, Final	Body weight, Average	Food intake	Dry-matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
3	29.07	27.70	28.39	2,810	2,697.86	7.66	19.21	[b] 0.712			10.85	[c] 0.382						
4	29.23	27.70	28.47	3,452	3,315.63	9.58	24.57	[b] .741			11.81	[c] .415						
5	36.30	35.13	35.72	4,119	3,952.66	11.62	18.66	[b] .472			11.91	[c] .333						
ALFALFA HAY ALONE																		
3	31.70	31.03	31.37	9,693	8,882.67	263.75	78.50	61.91	16.59	247.16	131.84	12.11	119.73	127.43	70	20	29	52
4	29.50	28.33	28.92	6,930	6,354.06	180.82	51.32	42.32	9.00	171.82	101.07	11.60	89.47	82.35	72	16	22	48
5	35.80	36.27	36.04	10,000	9,164.00	272.10	75.44	45.36	30.08	242.02	134.65	13.05	121.60	120.42	72	23	32	50
ALFALFA-HAY RATION [d]																		
3	32.30	33.67	32.99	10,000	9,404.00	157.30	76.67	64.04	12.63	144.67	46.68	12.87	33.81	110.86	51	22	42	77
4	28.87	29.43	29.15	7,000	6,582.80	110.11	45.43	38.90	6.53	103.58	41.92	11.25	30.67	72.91	59	21	35	70
5	37.23	38.27	37.75	10,000	9,404.00	157.30	64.58	48.62	15.96	141.34	60.53	14.76	45.77	95.57	59	20	35	68
LOW-NITROGEN RATION																		
3	31.07	29.10	30.09	3,000	2,854.80	7.26	19.00	[b] 0.666			11.83	[c] 0.393						
4	28.27	27.50	27.89	3,150	2,997.54	7.62	15.48	[b] .516			10.37	[c] .372						
5	37.53	37.43	37.48	3,990	3,711.24	9.43	20.03	[b] .540			15.72	[c] .419						

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[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

[d] Ration consisted of 58.8 percent of alfalfa hay, 5 percent of cellulose, 15.6 percent of starch, 15.6 percent of sugar, 3 percent of corn oil, and 2 percent of minerals (equal parts of steamed bone meal, ground limestone, and sodium chloride).

The writers believe that this is the case when alfalfa hay is fed alone with no additional energy supplements. Alfalfa hay fed alone in this third experiment furnished 18.6 percent of protein, on a dry basis, and approximately 52 percent of total digestible nutrients, whereas the supplemented ration contained 10.5 percent of protein and about 68 percent of total digestible nutrients. It seems quite possible that this wide difference may account for the differences in the biological value of alfalfa protein obtained by these two methods of feeding.

In this connection it is of interest to note that Sotola (*19*), in his studies of the biological value for sheep of the protein in alfalfa hay cut at various stages of maturity, pointed out the effect of a difference in plane of protein intake on the biological values of the protein in rations. On an average, the biological value of the protein in the hay cut at the three-fourths to full-bloom stage was 68, as compared with 61 for hay cut at earlier stages of maturity. Sotola concluded that the higher biological values for the later cut hay were "due more to a lower concentration of protein in the ration than to the number of the cutting."

It seems probable that the wide difference in the protein content of alfalfa stems and of alfalfa leaves was the reason Sotola (*21*) secured much higher values for the protein in the stems than in the leaves, when each was used as the only feed for sheep. In these studies Sotola found an average biological value of 64 for the protein in alfalfa stems containing 8.48 to 9.53 percent of protein, on a dry basis, and an average biological value of only 44 for the protein in alfalfa leaves containing 20.34 to 22.20 percent of protein.[4] Similarly, it seems that the wide difference in the plane of protein intake may have been the chief factor in causing the great difference that Sotola observed in the biological values of the protein in corn silage and in alfalfa hay when these feeds were each fed alone to sheep (*20*). In these studies, the average biological value for corn silage, which contained only 5.5 percent of protein on the dry basis, was 94, as compared with 56 for alfalfa hay containing 14.2 percent of protein.

It is obvious that no attempt was made to standardize the rations fed in this third experiment in regard to the protein and energy content. Instead, the object was to find out whether the excessive protein intake and relatively low energy intake as furnished by a ration of alfalfa hay alone would account for the low biological value of alfalfa protein for sheep reported by other investigators. This object was accomplished.

The results of these experiments show that alfalfa hay is probably not deficient in quality of protein for sheep when fed in a balanced ration as regards protein and total digestive nutrients, and that the biological values obtained may depend upon the particular system of feeding and experimentation employed.

These results are especially interesting since it has been assumed that sheep need larger amounts of cystine, the chief sulphur-containing amino acid, than other farm animals. This assumption is based on the fact that wool is high in this amino acid. Some investigations (*3*) have shown a probable deficiency of cystine in alfalfa hay. However, it is known that alfalfa hay is quite high in sulphur. If alfalfa hay is generally deficient in cystine, these results might possibly indicate that this amino acid can be synthesized somewhere in the body, perhaps by the micro-organisms in the digestive tract, from the other sulphur-containing compounds present in the hay.

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[4] Since this paper was submitted for publication the detailed report of these experiments appeared in the following publication: Jour. Agr. Research 40: 919–945, 1933. SOTOLA, J. THE NUTRITIVE VALUE OF ALFALFA LEAVES AND STEMS. In this it is stated with reference to the differences in the biological values obtained when alfalfa stems, alfalfa leaves, and entire alfalfa hay were fed separately as the only feeds to lambs, "the variations in values are attributed to the differences in protein concentration of the stem, leaf, and whole-hay rations."

SUMMARY

In nitrogen metabolism studies with five growing wether lambs, the digestibility, storage, and biological value of the proteins of alfalfa hay and clover hay, and of the proteins in the combination of each hay with corn, have been determined. These rations were equalized in energy content by the addition of cornstarch and canesugar in various amounts.

The average coefficients of apparent digestibility of protein were 50 for clover hay, 56 for alfalfa hay, 55 for the clover and corn combination, and 63 for the combination of alfalfa and corn. These results are statistically significant in showing that the protein of alfalfa is more digestible than that of clover and that the protein of the combination of each hay with corn has a higher digestibility than the protein of either hay when fed as the only source of protein. If allowance is made for the metabolic fecal nitrogen and the true digestibility of the ingested protein calculated, the same relative differences in digestibility were also shown.

No marked differences were observed in the percentage of protein stored from the different rations. However, the difference in the protein storage from the combination of alfalfa and corn and the storage either from the alfalfa-hay or the clover-hay ration, were sufficient to indicate that these differences were possibly significant from a statistical standpoint.

Very little difference was found in the efficiency with which the different proteins were utilized for growth and maintenance, as measured by the biological value. The average of the biological values was 81 for clover protein, 79 for alfalfa protein, 80 for the protein in the combination of clover and corn, and 77 for the protein in the combination of alfalfa and corn.

In an experiment with 3 lambs, alfalfa hay fed with no addition of starch and sugar gave biological values averaging 50, whereas after the addition of starch and sugar to the ration, the biological value was 72. These results indicate the influence which the energy content and the plane of protein intake of the ration may have on the biological value of protein; they also show that values obtained may depend upon the method of experimentation used for their determination.

These experiments show that alfalfa hay is probably not deficient in quality of protein for sheep when fed in a balanced ration.

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RELATION OF LENGTH OF DAY TO GROWTH OF TIMOTHY[1]

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INTRODUCTION

Since 1920, when Garner and Allard[2] first announced their discovery of the principle of photoperiodism, many investigators have published results of studies of this phenomenon as it applies to many kinds of plants.[3]

In some plants, earliness and lateness are not the only characteristics that are affected by changes in the length of day. It has been shown that in timothy (*Phleum pratense* L.) not only time of blooming but also elongation of the internodes of the stems and the number of elongated internodes per culm are affected by the length of day.[4]

In a preliminary test conducted in 1930 it was found that timothy plants that bloom and mature early when grown under natural conditions respond to days artificially made of uniform lengths in a different way from plants that are later in their development. This discovery suggested the desirability of conducting an experiment for the purpose of determining the responses of timothy plants that bloom and mature at different times to days that range in length by uniform gradations from relatively short to relatively long.

When plants propagated vegetatively from a single original timothy plant are grown at stations in different latitudes, it has been found that in the Northern Hemisphere the season for blooming of these plants does not progress from south to north at the uniform rate per day of one quarter of a degree, as predicted in Hopkins' bioclimatic law.[5] On the contrary, the plants bloom relatively later at the southern stations and relatively earlier at the northern stations than this law prescribes.[6] One possible explanation for this is that the development of the plants in the South during early spring is delayed by the relatively short days that occur in southern latitudes, and in northern latitudes the development is hastened by the relatively long days that occur during late spring and early summer.

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[1] Received for publication Nov. 17, 1933, issued June 1934

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MATERIAL AND METHODS

For this investigation selected timothy plants were used. These plants are referred to as strains of timothy. All the strains listed in tables 2 and 3, except the last four, were selected from plants grown from seed of American origin; the last four originated from seed from northern Europe and are later than plants ordinarily occurring in meadows in the United States. These strains have been arranged in a series, according to the dates when the first florets bloomed on plants growing with natural illumination at Washington, D.C., in 1931. They represent uniform gradations from the earliest to the latest. The experiment was conducted at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C.

On December 16, 1930, each of several plants was divided, and each subdivision, bearing the same number that had been assigned the original plant, was placed in a 3-inch pot. On January 26, 1931, each plant was transferred to a 4-inch pot. The plants were grown in a cool greenhouse (50° to 55° F.) until February 18, 1931, when they were removed to coldframes built upon trucks on tracks. These trucks were moved out of doors for 10 hours each day until the final tests under the various lengths of day began; during the remainder of the time they were kept in dark houses.

After the experiment was begun, plants of each strain were grown under all or part of the following numbers of hours of illumination each day: 10, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, and 18. In addition, one or more plants of each strain, used as controls, were grown under the natural lengths of day.

The length from sunrise to sunset of the longest day occurring at Washington is 14 hours and 54 minutes. The plants grown with uniform lengths of day less than this maximum were placed in metal cans or buckets on trucks, which were moved into ventilated dark houses for that part of each day during which the plants received no illumination. The plants grown with 15 hours or more of illumination each day were placed out of doors, where the length of day was extended as desired by means of electric lights placed over the plants.

The plants that received 14.5 hours or less of illumination were out of doors each day for the periods indicated in table 1, from the date the test began until the final records were obtained.

TABLE 1.—*Time during which timothy plants receiving illumination for 10 to 14.5 hours daily were out of doors*

Date when test began	Length of period of illumination each day	Hours during which plants were out of doors	Date when test began	Length of period of illumination each day	Hours during which plant were out of doors
	Hours			*Hours*	
Apr. 11	10	From 6 a.m. to 4 p.m.	May 18	13.5	From 5 a.m. to 6:30 p.m.
Do	12	From 6 a.m. to 6 p.m.	Do	14	From 5 a.m. to 7 p.m.
Do	12.5	From 6 a.m. to 6.30 p.m.	Do	14.5	From 5 a.m. to 7:30 p.m.
Do	13	From 5 a.m. to 6 p.m.			

Artificial light was required in order to obtain constant illumination for 15, 16, 17, and 18 hours each day, periods representing approximately the maximum lengths of day for the latitudes 41°, 48°, 53°, and 57°, respectively. This added illumination was furnished for each group of plants by four 200-watt gas-filled tungsten lights with reflectors, built according to standard specifications. These lights were placed at each corner of a square of such dimensions that the distance from center to center of the lights was 3 feet. The lights, which were kept about 2 feet above each group of plants, were arranged on an adjustable frame so that they could readily be raised as the plants increased in height. Each set of plants was screened by partitions high enough to cut off the direct rays of its group of lights from all the other sets of plants.

In order to maintain illumination for a uniform period each day, it was necessary to use electric lights for different lengths of time. Constantly decreasing daily periods of artificial light were required up to the midsummer solstice, and constantly increasing periods of light after the solstice. Four electric time switches were used to control the required decrements or increments of artificial light, and the necessary changes were made each day. The tests under all four lengths of day, from 15 to 18 hours, began April 11. The lights were turned on at 5 p.m., and were kept on for different periods, so that the different groups of plants were illuminated for 15, 16, 17, and 18 hours, respectively, from sunrise each day.

EXPERIMENTAL RESULTS

EFFECTS OF DIFFERENT LENGTHS OF DAY ON DIFFERENT PHASES OF GROWTH

Insofar as vegetative growth is concerned, the plants thrived under all lengths of day within the range of this experiment. There were, however, great variations (1) in the time when the heads emerged from the enclosing leaf sheaths, (2) in the time of occurrence of the flowering process, (3) in the characteristics of the stems, and (4) in the development of the stems.

Time When Heads Emerge from Enclosing Leaf Sheaths

The date of emergence of the earliest head of each plant from within the enclosing leaf sheath is shown in table 2.

In general, the later in the season the heads appeared on plants grown with natural illumination, the greater was the number of hours of illumination required for the development of the inflorescences on plants of the same strain grown with artificial light. Plants of the 2 strains that were earliest under natural conditions produced inflorescences when subjected to only 10 hours of light each day. Plants of 1 of the 4 strains that were latest under natural illumination required day lengths of 14.5 hours, while none of the plants of the other 3 strains of this group produced inflorescences under less than 15 hours of illumination daily. Plants of the other strains had a minimum light requirement of between 10 and 14.5 hours each day for the development of inflorescences; in general, the minimum number of hours of illumination required tended to increase gradually as the time of heading became later on the plants of these strains when grown under natural conditions.

As the number of hours of illumination each day increased, the date of heading on the plants of any strain gradually became earlier until the optimum day length had been attained; after this, even with continued increase in the length of day, the date of heading remained practically the same. This statement may be illustrated by the

TABLE 2.—*Dates in 1931 when the first heads appeared on plants of different strains of timothy, grown with natural daylight from 10 hours to full length of day each day and with added artificial light to obtain illumination of 15 to 18 hours daily, near Washington, D.C.*[a]

Strain no.	Date of emergence [b] of first heads in plants grown with indicated hours of illumination											
	Full day	10	12	12.5	13	13.5	14	14.5	15	16	17	18
19456	May 20	June 22	May 18	May 18	May 20	(c)	(c)	(c)	(c)	(c)	(c)	(c)
19457	May 25	June 23	June 5	June 3	June 3	May 22	(c)	(c)	(c)	(c)	(c)	(c)
19458	May 26	------	------	June 15	June 16	(c)	(c)	(c)	(c)	(c)	(c)	(c)
15092	May 28 May 26	------ ------	June 27 June 24	June 8 June 10	June 15 do	May 28 do	May 28 May 26	May 28 May 25	May 18	May 15	May 13	May 13
11902	June 1	------	July 24	July 1	June 25	June 3	June 1	June 1	May 20	May 18	May 14	May 18
6127	June 8 June 10 June 12	------	------	------	------	June 18 June 27	June 15	June 8	May 25	May 20	May 19	do
6743	June 8 June 6	July 20	------	June 26	June 27	June 11	June 8	June 4	do	June 1	do	May 15
11966	June 13 June 16	------ ------	------	------ ------	July 22 ------	July 3 June 23	June 22 June 15	June 17 June 13	May 29	May 21	do	May 16
9220	June 18	------ ------	Aug. 3 July 31	July 21 July 11	Aug. 3 July 13	July 7 July 13	June 26 June 30	June 19	June 1	May 19	May 18	May 18
12421	June 19	------	------	------	------	July 14	do	June 22	June 5	May 25	May 20	May 21
15485	June 25 June 26	------	------	------	------	July 22	July 9	June 30	June 8	May 26	May 22	May 22
19416	June 27 June 30	------	------	------	------	------	July 10	June 27	do	do	May 21	May 21
15445	July 3 July 7	------	------	------	------	------	------	------	June 20	June 1	May 26	May 26
19459	July 3 July 6 July 11	------	------	------	------	------	------	------	June 13	May 29	May 23	May 21
19460	do July 15	------	------	------	------	------	------	July 20	June 16	June 1	do	May 23
19461	July 13 July 17	------	------	------	------	------	------	------	June 18	June 3	May 25	May 25

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[a] To obtain daily exposures of 10 to 14.5 hours required keeping the plants in a darkened house for a certain number of hours each day; to obtain daily exposures of 15 to 18 hours required the use of artificial light to supplement the normal length of day.

[b] Leaders indicate that no heads emerged under the period of illumination specified; more than 1 date of emergence or line of leaders merely indicates that the test included 2 or more sets of plants.

[c] No plants were grown under this period of illumination.

records of the plants of strain no. 9220 (table 2). Under natural conditions, the first head appeared on the control plant on June 18. On the plant grown with 10 hours of light each day, no inflorescence developed; on that grown with 12 hours of light the tip of the first head appeared on July 31. With increasing length of day the time of heading gradually became earlier, until with 16 hours of illumination the time of heading occurred on May 19. When exposed to 17 and 18 hours of light each day, the heads of the plants appeared on May 18, practically the same date as on the plant grown under a 16-hour day.

TIME OF FLOWERING

The relative dates at which the first florets appeared on plants of the different strains grown with different periods of illumination corresponded, in a general way, to the relative dates at which the first heads had appeared on the same plants about 12 to 15 days earlier.

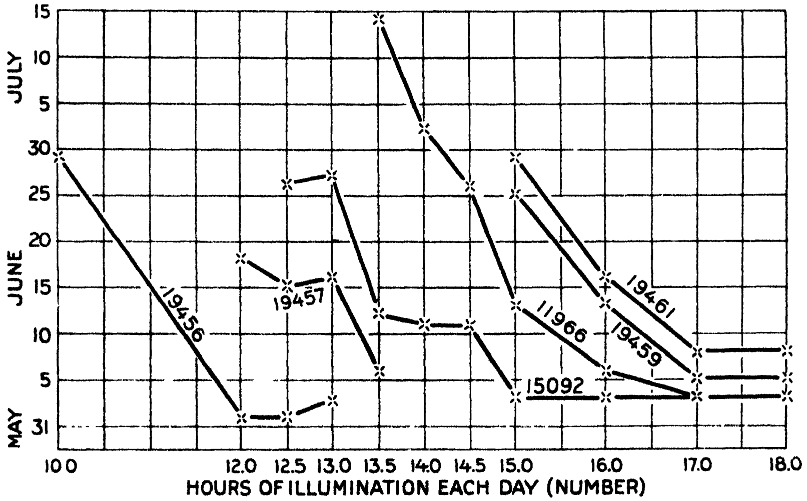

FIGURE 1. Dates in 1931 when the first florets bloomed on different timothy strains (indicated by numbers), grown under various constant daily periods of illumination.

In plants that under natural conditions range gradually from very early to very late the response to days of different lengths, as indicated by the time of flowering, is quite consistent (table 3). On the control plant of strain no. 19456, the first florets bloomed on June 3; this was the earliest plant to bloom under natural conditions. On the plant of this strain grown with 10 hours of light each day, the first florets bloomed on June 29. As the date of flowering became later on plants of the different strains grown under natural conditions, the minimum number of hours of illumination under which any florets bloomed gradually increased, until, in the case of strain no. 19461, which was the latest to bloom under natural illumination, no florets appeared on plants grown under days of uniform length of less than 15 hours.

As the length of day increased, the dates on which the florets on the plants of any particular strain bloomed gradually became earlier up to the optimum period of illumination, after which there was no further increase in earliness. This tendency is shown by the records in table 3 and the curves in figure 1. Under natural conditions, the first florets

TABLE 3.—*Dates in 1931 when the first florets opened on plants of different strains of timothy, grown with natural daylight from 10 hours to full daylight each day and with added artificial light to obtain illumination of 15 to 18 hours daily, near Washington, D.C.*[a]

Strain no.	Date of first flowering [b] of plants grown with indicated hours of illumination											
	Full day	10	12	12.5	13	13.5	14	14.5	15	16	17	18
19456	June 3	June 29	June 1	June 1	June 3	(c)	(c)	(c)	(c)	(c)	(c)	(c)
19457	June 8	-----	June 18	June 15	June 16	June 6	(c)	(c)	(c)	(c)	(c)	(c)
19458	June 11	-----	-----	June 27	June 26	(c)	(c)	(c)	(c)	(c)	(c)	(c)
15092	June 12 June 13	----- -----	July 14 -----	June 22 June 26	June 27 June 29	June 12 do.	June 11 June 13	June 11 June 12	June 3	June 3	June 3	June 3[a]
11902	June 17 June 18	-----	Aug 4	July 13	July 10	June 19	June 16	June 18	June 6	do.	do.	Do.
6127	June 19 June 23	----- -----	----- -----	----- -----	----- -----	June 29 July 6	June 26	June 22	June 8	June 5	do.	Do.
6743	June 22 June 23	-----	-----	-----	July 17	June 25	June 29	June 19	June 12	-----	do.	Do.
11966	June 26 June 30	-----	-----	-----	----- -----	July 14 July 20	July 2 July 6	June 26 June 29	June 13	June 6	do.	Do.
9220	June 29 July 2	----- -----	----- -----	----- July 18	----- Aug. 1	----- July 23	do. July 13	July 2	June 16	June 5	do.	Do.
12421	June 30	-----	-----	-----	-----	-----	do.	July 3	June 15	June 8	June 5	June 4
15485	July 6 July 7 July 9	-----	-----	-----	-----	-----	July 20	July 9	June 20	do.	do.	June 5
19416	July 11 -----	-----	-----	-----	-----	-----	-----	July 11	June 22	do.	do.	June 4
15445	July 14 July 20	-----	-----	-----	-----	-----	-----	-----	July 2	June 16	June 12	June 8
19459	July 17 July 20	-----	-----	-----	-----	-----	-----	-----	June 25	June 13	June 5	June 5
19460	July 22 July 25	-----	-----	-----	-----	-----	-----	July 29	June 29	June 16	June 8	June 4
19461	do. July 27	-----	-----	-----	-----	-----	-----	-----	do.	do.	do.	June 8

[a] To obtain daily exposures of 10 to 14.5 hours required keeping the plants in a darkened house for a certain number of hours each day, to obtain daily exposures of 15 to 18 hours required the use of artificial light to supplement the normal length of day

[b] Leaders indicate that no florets opened under the period of illumination specified: more than 1 blooming date or line of leaders merely indicates that the test included 2 or more sets of plants.

[c] No plants were grown under this period of illumination.

on plants of no. 11966 bloomed on June 26. When the plants were grown under days 10 to 13 hours long, no florets bloomed; when they were grown with 13.5 hours of light, florets began to bloom on July 14. As the length of day increased, the date of blooming became gradually earlier up to days of 17 hours, under which the earliest florets bloomed on June 3. When the length of day was increased from 17 to 18 hours, the date of earliest bloom remained the same, June 3. There was a similar response to days of different lengths in the plants of other strains.

The later the plants of any strain of timothy grown under natural conditions produce inflorescences and florets in bloom, the greater is the length of day required for normal development when the plants are grown with days of various constant lengths. This statement is illustrated in figures 2 to 6, which show the plants as they appeared on July 1. The plants of strain no. 19456, the earliest under natural conditions, when grown with 12 hours of light each day produced inflorescences on which florets had bloomed before July 1. The plants of strain no. 15092 required a day of about 13.5 hours, and those of strain no. 6127 a day of 14 hours for normal growth. The plants of no. 19461, which was the latest strain used in this experiment, required a day of 16 hours for the production of normal culms with inflorescences.

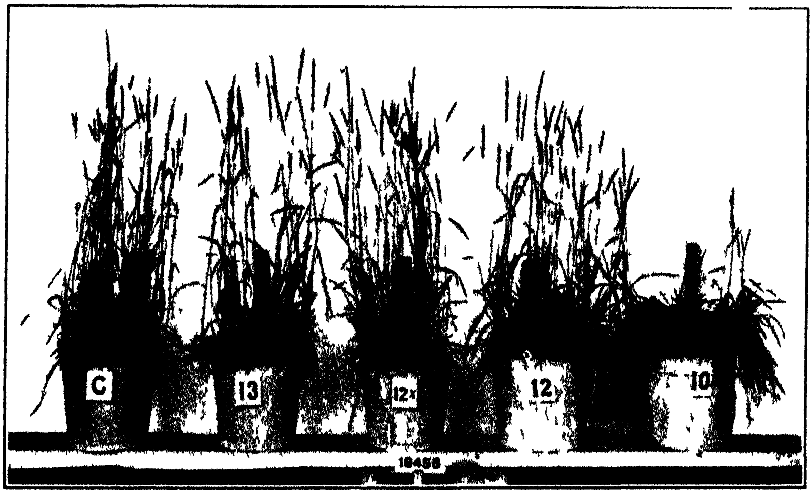

FIGURE 2.—Plants of timothy strain no. 19456, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

If the natural length of day is too short for the development of culms and inflorescences of any timothy plant, it may be artificially increased, by means of electric light, to the daily number of hours of illumination which the plant requires. This is illustrated in figure 7, which shows plants of strain no. 19461 grown with natural illumination and with 15, 16, 17, and 18 hours of light each day. On the plants grown with natural illumination no inflorescences appeared until July 13, whereas on the plants grown with 16, 17, and 18 hours of light each day, normal culms and inflorescences had developed before June 25.

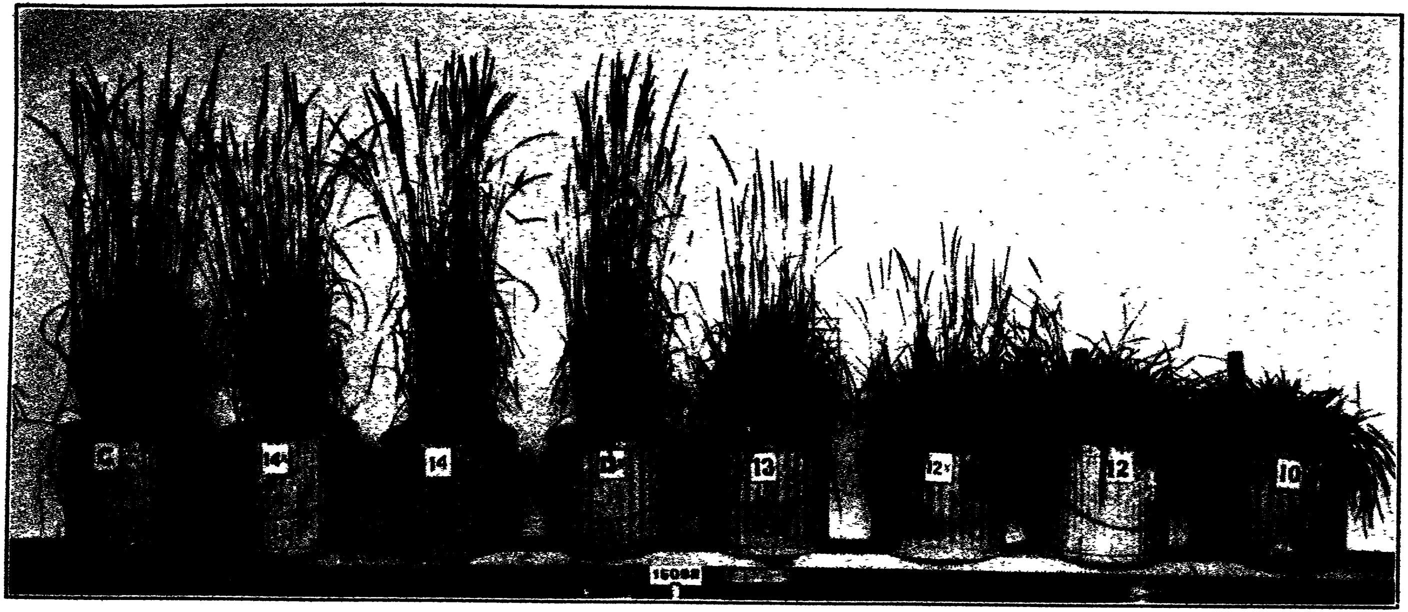

FIGURE 3.—Plants of timothy strain no. 15092, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931

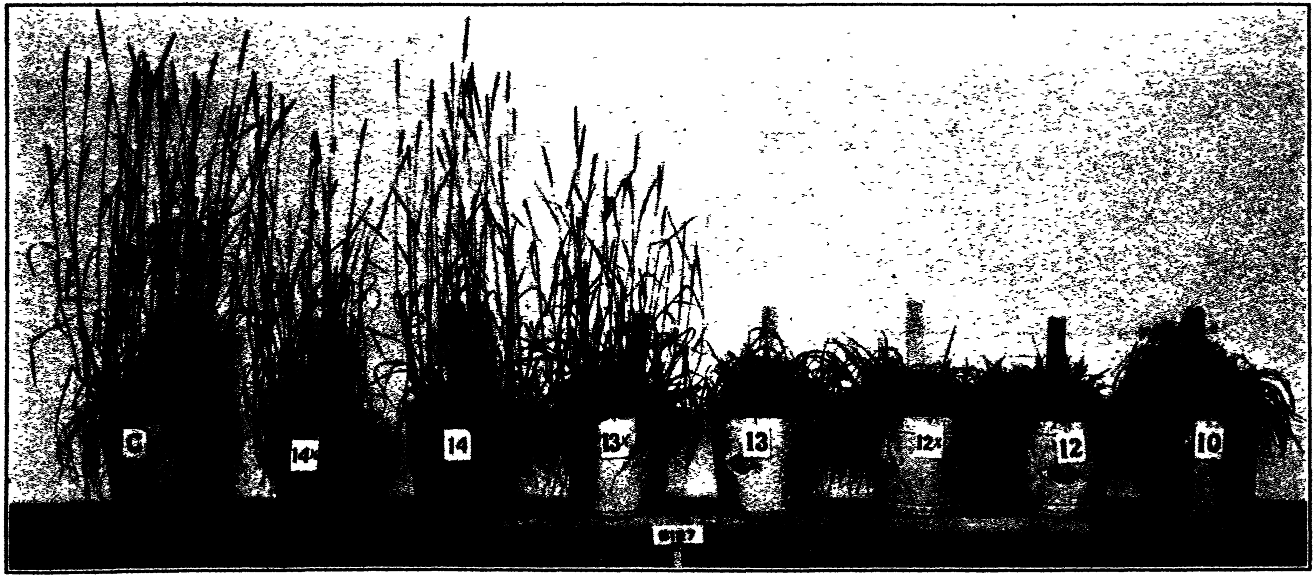

FIGURE 4.—Plants of timothy strain no 6127, grown under different lengths of day, as indicated by number of hours on containers The control plant (*C*) was grown under natural length of day Photographed July 1, 1931.

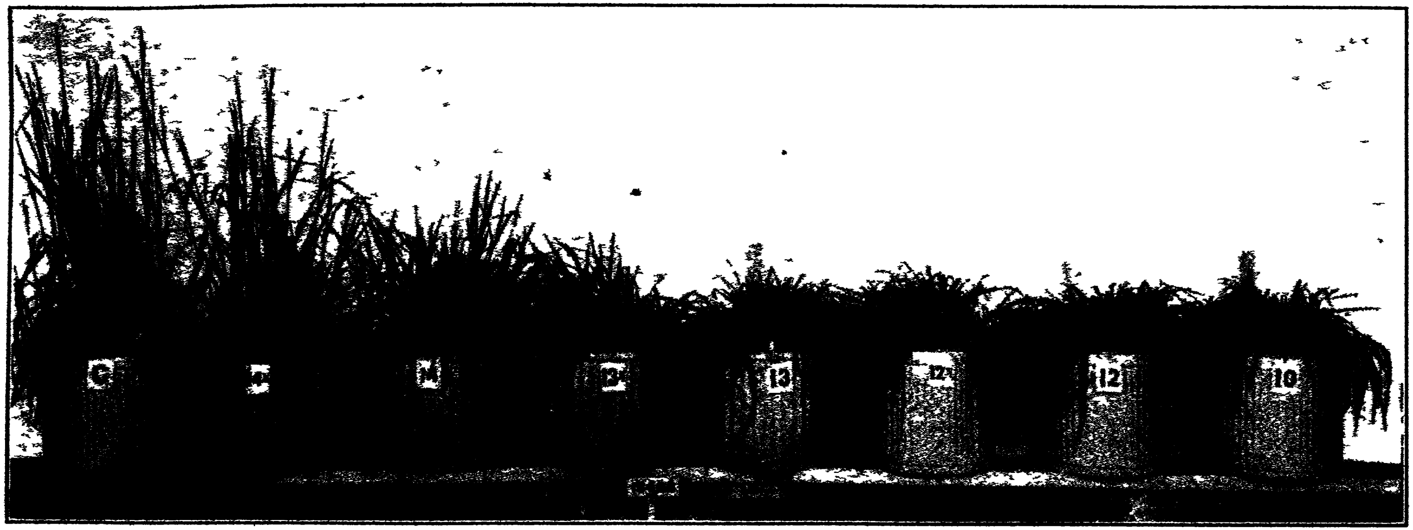

FIGURE 5.—Plants of timothy strain no. 15485, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

CHARACTERISTICS OF STEMS

The stems of plants which are grown with sufficiently long periods of illumination each day to enable the inflorescences to develop in a normal manner and on which the florets bloom at a normal time usually grow upright. In this experiment the plants that were grown under days too short for normal development of the inflorescences were commonly characterized by stems which were declined or which were more or less procumbent at the base, sometimes bearing inflorescences with proliferations. Figures 3 and 4 show that the plant of strain no. 15092 grown with 12.5 hours of illumination and the plant of strain no. 6127 grown with 13.5 hours of illumination daily had a tendency to a spreading habit of growth owing to the declined position of the stems.

On plants of all strains except those that were earliest under natural conditions, no elongation of the stems occurred under the shortest periods of illumination. Thus, on the plants of no. 6127 (fig. 4), there was no elongation of stems under 10 to 13 hours of illumination daily; under 13.5 hours of illumination, elongation occurred although the stems were somewhat declined; on the plants grown under 14 and 14.5 hours of light each day and those grown under natural illumination, the stems grew in an upright position.

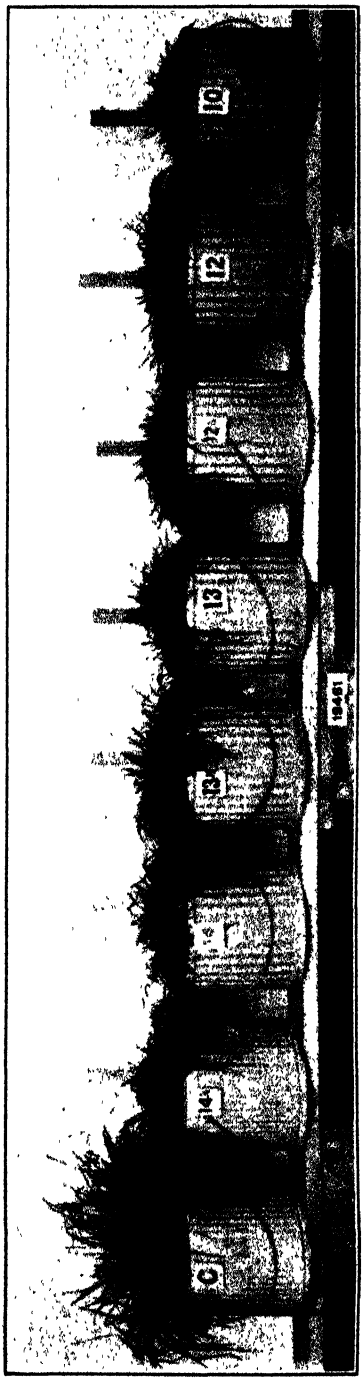

FIGURE 6.—Plants of timothy strain no. 19461, grown under different lengths of day, as indicated by number of hours on containers. The control plant (*C*) was grown under natural length of day. Photographed July 1, 1931.

LENGTH OF STEMS

At the time of the first blooming of the florets of a timothy plant, the stems on which the inflorescences are borne have attained only a part of their final length.[7]

The data obtained in this experiment show that when the first florets bloomed the actual length of the stems varied according to the number of hours of daily illumination under which the plants had been grown. The records show that generally, as the length of day increased, the length of the stems of the plants of any strain on the date when the first florets bloomed gradually increased to the maximum. After this maximum had been reached, the length of the stems was not essentially changed by continued increase in the length of day. For example, when the plants of timothy no. 11902 were grown with 12 hours of light, the longest stem was 16 inches at the time the florets began to bloom; as the length of day increased, the length of stem at the time the first florets bloomed also increased by fairly uniform steps to a maximum length of 41 inches on the plant grown with 15 hours of light each day. On the plants grown with 16, 17, and 18 hours of illumination daily, the length of the longest stem at the time the first florets bloomed was either 40 or 41 inches, practically the same length as on the plant illuminated for 15 hours each day. Table 4 and figure 8 show the average length of the longest stems of the plants in groups of early, medium, and late strains of timothy grown under different lengths of day.

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[7] EVANS, M. W. Pp. 10–13. (See footnote 4.)

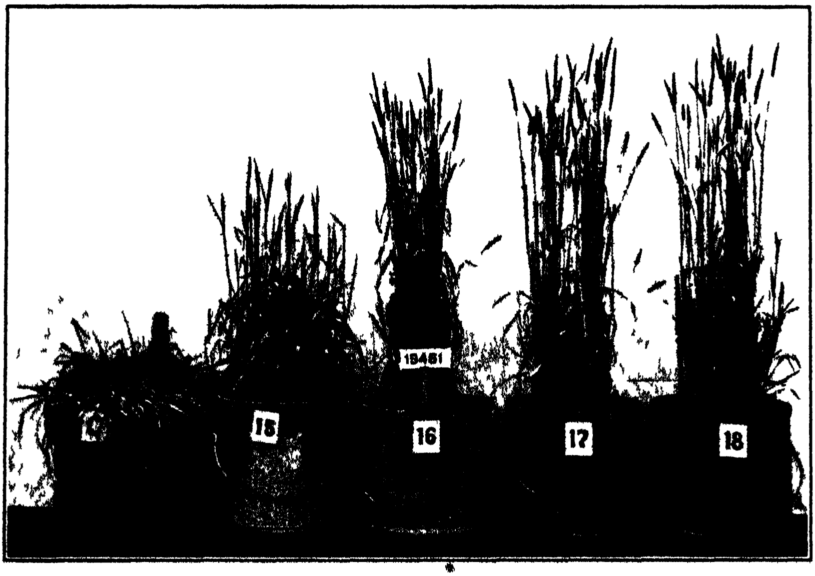

FIGURE 7.—Plants of timothy strain no. 19461 grown under natural length of day (*C*) and with days longer than normal, as indicated by number of hours on containers. This strain of timothy is the latest one (when grown under natural conditions) that was used in this experiment. Photographed June 25, 1931.

RESULTS OF CONTINUED GROWTH UNDER DAYS TOO SHORT FOR DEVELOPMENT OF CULMS AND INFLORESCENCES

In relation to those plants which were grown under days of short lengths and on which only vegetative growth had taken place prior to July 1, the question arises as to whether continued growth for a longer time under the same conditions would have resulted in the development of culms and inflorescences. The results obtained with the plants of strain no. 15485 are fairly typical and may be used to illustrate the general behavior, in this respect, of the plants of all the strains of timothy studied. Figure 9 shows the condition of the plants of this strain on August 10, 6 weeks after the plants in figure 5 were photographed. On plants upon which partial development of culms occurred before July 1, there was a somewhat more advanced development on August 10. On plants that were grown with 10, 12, 12.5, and 13 hours of light each day, and on which only vegetative growth had taken place prior to July 1, no further development occurred as a result of 6 weeks of added growth. If timothy plants are grown, therefore, under days too short for the formation of culms and inflorescences these will not be formed even though the period of growth under the same light conditions is extended.

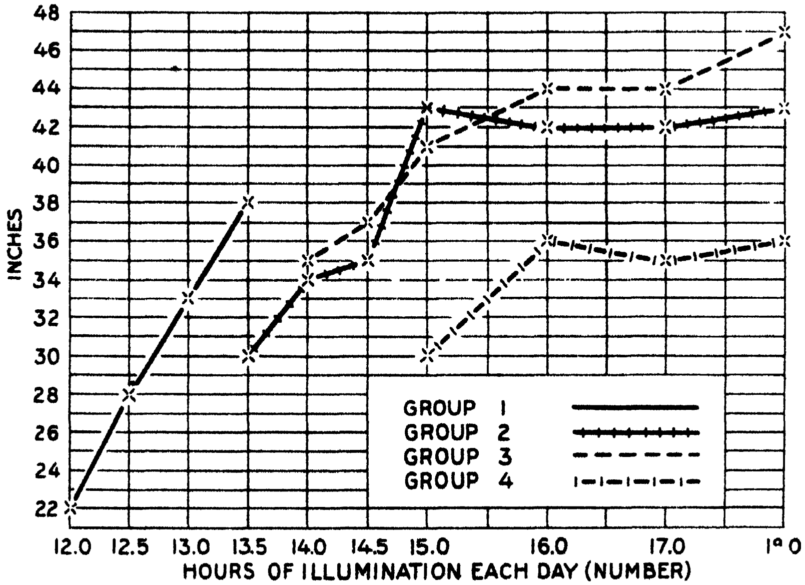

FIGURE 8.—Average length of the longest stem (in inches) of early (group 1), medium (groups 2 and 3) and late (group 4) timothy plants grown under various constant daily periods of illumination

TABLE 4.—*Average length (inches), on the date when the first florets bloomed, of the longest stem of timothy plants grown under various constant daily periods of illumination and grouped according to relative earliness* [a]

Group	Average length (inches) of the longest stem grown under indicated hours of illumination daily [b]											
	Natural	10	12	12.5	13	13.5	14	14.5	15	16	17	18
1	38	...	22	28	33	38	([c])	([c])	([c])	([c])	([c])	([c])
2	34	...	...	...	...	30	34	35	43	42	42	43
3	39	...	...	...	...	...	35	37	41	44	44	47
4	27	...	...	...	...	...	...	...	30	36	35	36

[a] When grown under normal conditions, plants of group 1 (nos. 19457 and 15092) were the earliest strains of timothy used in this experiment; those of group 2 (nos. 11902, 6127, and 11966) were the next earliest, those of group 3 (nos. 9220, 12421, and 15485) were next, and those of group 4 (nos. 15445, 19459, 19460, and 19461) were the latest

[b] Leaders indicate that no average was available, because flowering either did not take place or some of the strains failed to flower under the indicated period of illumination.

[c] No average given because there were no plants of no. 19457.

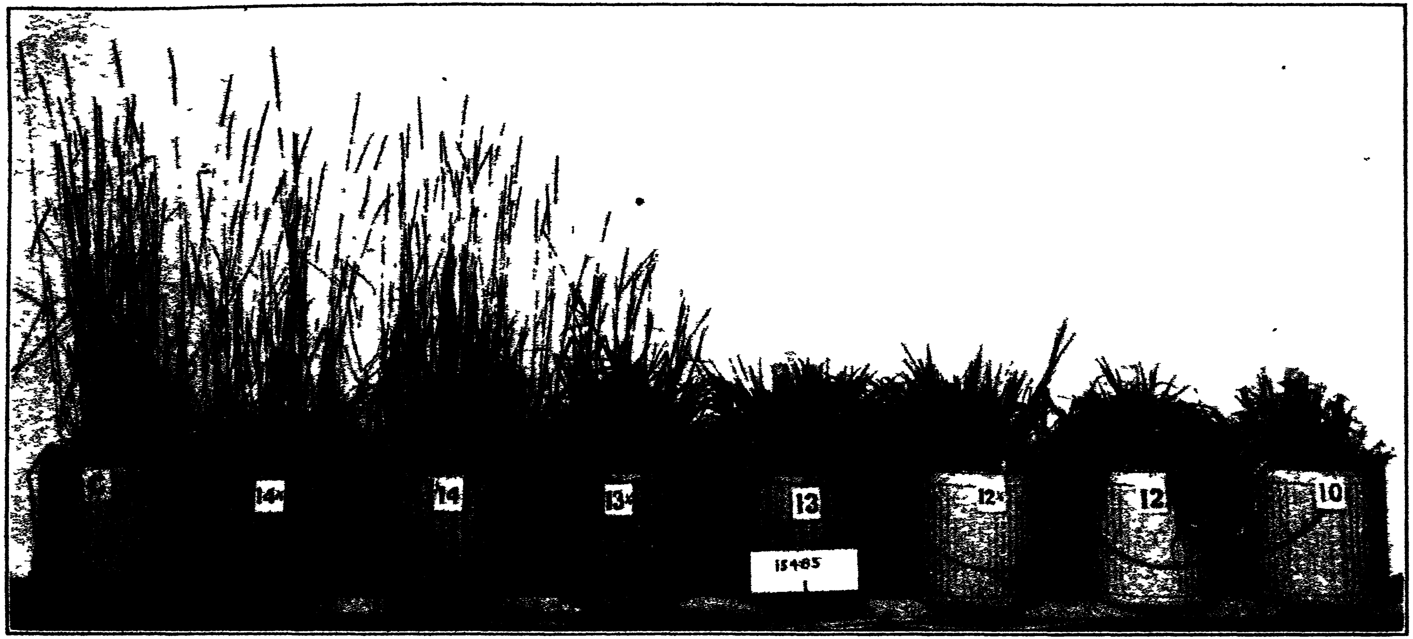

FIGURE 9 —Plants of timothy strain no 15485, grown under different lengths of day, as indicated by number of hours on containers The control plant (*C*) was grown under natural length of day Photographed August 10, 1931 Some increase in the length of the stems on plants illuminated for 13.5 hours or more each day since July 1 may be observed by comparing this photograph with figure 5 Under 13 hours or less of light each day, no growth in length of the stems had taken place

COMPARISON OF EFFECTS OF NATURAL AND ARTIFICIAL ILLUMINATION

The length of the longest day at Rosslyn, Va., where this experiment was conducted, is 14 hours and 54 minutes, or 14.9 hours. Tables 2 and 3 show that in most strains of timothy the heads appeared and the florets bloomed at approximately the same time on plants illuminated for 14.5 hours each day as on the control plants; on the plants grown under days 15 hours long, the heads appeared and the florets bloomed earlier than on the plants grown with natural illumination.

EARLINESS AND LATENESS IN TIMOTHY AN ADJUSTMENT TO DIFFERENT LENGTHS OF DAY OCCURRING AT DIFFERENT TIMES

In timothy, as in some other plants,[8] the earliness or lateness of different strains is evidently a matter of the adjustment of the plants to length of day. In figure 10 the strains of timothy are arranged according to the dates when the first florets bloomed on the plants grown with natural illumination; the first heads had appeared on these plants in practically the same order. Figure 10 also shows the dates on which the first heads appeared and the first florets bloomed on plants of most of these strains when grown with 18 hours of light each day, the maximum length of day employed. When the length of day was increased to 18 hours, plants of those strains that are late under natural conditions produced heads and florets in bloom almost as early as plants of those strains that are early under natural conditions (fig. 10).

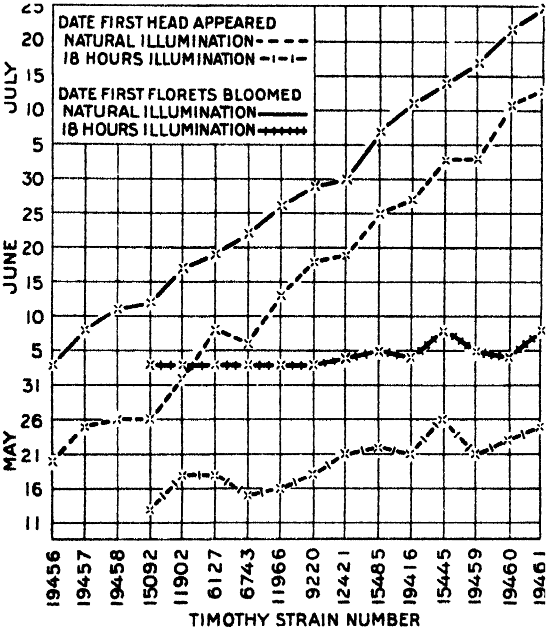

FIGURE 10.—Dates when the first head appeared and the first florets bloomed on timothy plants grown near Washington, D.C., under natural illumination and under 18 hours of illumination each day. Note that plants of those strains that are late under natural conditions in the latitude of Washington, D.C., when grown under 18 hours of daily illumination produced heads and florets in bloom nearly as early as plants of those strains that are early under natural conditions in the same locality.

The results of this experiment indicate that in any locality some timothy plants produce inflorescences and florets in bloom earlier than other plants because they are adapted to relatively short days. On those plants that are adapted to longer days, the appearance of the heads and the flowering process are delayed until the days lengthen.

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[8] GARNER, W. W., and ALLARD, H. A. PHOTOPERIODIC RESPONSE OF SOYBEANS IN RELATION TO TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS. Jour. Agr. Research 41: 719-735, illus. 1930.

SUMMARY

At the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D.C.) there were grown under days of different lengths strains of timothy plants that when grown under natural conditions constitute a series ranging by fairly uniform gradations from very early to very late in the time their inflorescences appear, florets bloom, and seeds mature. In addition to the control plants grown under natural illumination, plants were grown with the periods of illumination artificially regulated by means of dark houses and electric lights to days 10, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, and 18 hours long.

Insofar as vegetative growth is concerned, the plants grew well under all lengths of day within the range used in this experiment. There were great variations, however, with respect to the time of emergence of the heads from the enclosing leaf sheaths, the flowering process, and the characteristics and development of the stems.

The later the plants of different strains of timothy produce inflorescences and florets in bloom when grown under natural conditions, the greater is the length of day required for normal development when the plants are grown under days of different uniform lengths.

As the length of day is gradually increased above the minimum under which development of culms with inflorescences occurs, the stems lengthen and the time of the appearance of the inflorescences and the blooming of the florets gradually is shortened up to an optimum length of day. If the length of day is increased above this optimum, there is little added effect—at least up to 18 hours, the greatest length of day under which plants were grown in this experiment.

If the length of day is too short for the development of culms and inflorescences within the time that these processes ordinarily occur, continued growth of the plants for a longer time under the same length of day will not induce their development.

With a day of 14.5 hours, the timothy plants developed at about the same time as plants grown under the natural lengths of days occurring at Washington, D.C., which gradually increase up to a maximum of 14.9 hours at the summer solstice and then gradually decrease. When plants were grown under a day 15 hours long, they developed earlier than the control plants.

In timothy the earliness or lateness of different strains is evidently chiefly a matter of the adjustment of the plants to the lengths of day. The results of this investigation indicate that, in any locality, some timothy plants produce inflorescences and florets earlier than others because they are adapted to relatively short days. On those plants that are adapted to longer days, the appearance of the heads and the flowering process are delayed until, with the advancement of the season, the days increase to the lengths which these plants require for development.

DISTRIBUTION OF OXYGEN AND CARBON DIOXIDE IN MUSHROOM COMPOST HEAPS AS AFFECTING MICROBIAL THERMOGENESIS, ACIDITY, AND MOISTURE THEREIN [1]

By Edmund B. Lambert, *associate pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry,* and A. C. Davis, *assistant entomologist, Division of Truck Crop and Garden Insects, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

At the present time stable manure is the medium almost universally used for cultivating the common mushroom (*Agaricus campestris* L.). Growers have learned from experience that a period of composting is necessary before the manure is suitable for making up into beds. A rapid fermentation during which comparatively high temperatures are generated is apparently desirable. In general the rate of decomposition of the manure and the suitability of the finished compost for mushroom growing seem to be largely dependent on the size and shape of the compost heap, its height and compactness, the quantity of water added during turning, the thoroughness of the mixing, and the number of days between turnings. These factors probably influence the condition of the finished compost primarily by establishing in the compost heaps conditions of aeration, moisture, and temperature, which in turn establish the trend of the development of the microbial and insect population of the heaps.

The studies described in this paper were undertaken in order to improve composting practice by learning something of the distribution and interaction of these physical and biological factors in typical mushroom compost heaps. At first the writers were concerned principally with recording the temperature, aeration, moisture content, and acidity in all parts of standard mushroom compost heaps. As the observations progressed it became apparent that conditions are radically different in different parts of compost heaps and that the factors of temperature, moisture, and acidity are dependent on aeration, presumably through its effect on microbial activity, in a roughly predictable manner. From these observations an attempt has been made to derive principles that will give the experimenter an approximate conception of the conditions of aeration, temperature, moisture, and acidity to be expected in all parts of stable manure composted as for mushroom culture in heaps of any size or shape.

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[1] Received for publication Nov. 28, 1933, issued June, 1934.

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METHODS

Ordinary soil thermometers were used for taking temperatures in compost heaps. After the metal tips of these thermometers are heated the instruments may be removed from the compost and read before the mercury begins to fall. For readings at depths greater than 1 foot it was necessary to add extensions to the handles. Complete immersion of these thermometers seems to cause little difference in the readings, and this may be determined and a correction applied. The use of ordinary thermometers of any type is not desirable for three reasons: (1) The fall of the mercury is very rapid once the instruments have been removed from the manure and it is consequently very difficult to take accurate readings; (2) in drawing the thermometers from the bottom of the heaps through the warmer interior the readings change materially; and (3) it is necessary to punch holes in the heaps larger than the diameter of the thermometers in order to drop them in, thus admitting currents of air that frequently change the temperature.

In taking temperatures the thermometers were placed at horizontal and vertical intervals of 6 inches or 1 foot in the heap, depending on the time available and the accuracy desired. After each reading the thermometers were cooled to about 80° F., put into the same holes, and shoved down to the next level. The readings obtained were recorded on crosshatched paper, and the temperature contours were filled in later.

Samples of air from within the compost heaps were obtained by means of a metal tube having a sharply pointed end. Just behind the point four holes were bored for the air to enter. This tube was connected with rubber hose to an Orsat gas-analysis apparatus and could be thrust into the pile at any point for sampling. The air was removed from the tubing before each sample was taken. Samples from within the heap were passed first through a solution of potassium hydroxide to absorb the carbon dioxide and then through alkaline pyrogallol to absorb the oxygen. The probable error of the Orsat apparatus under these conditions seemed to be about 0.2 percent. At times the error of sampling was probably several times this figure. A possible source of error is recognized in the presence within the compost heaps of gases, other than carbon dioxide and oxygen, that might be soluble in potassium hydroxide or alkaline pyrogallol. But in all probability the presence of such gases in small quantities would have little bearing on the problem under consideration and would not affect the evidence or the conclusions in any way.

The hydrogen-ion concentration of samples from different parts of the compost heap was determined with a portable potentiometer by the quinhydrone method. The determinations were made in the field so that the samples were tested only a few minutes after they were removed from the compost heap. There is a tendency for the readings of manure samples to drift toward the alkaline side after the quinhydrone is added. To equalize this effect all samples were allowed an equal period (4 minutes) between the adding of the quinhydrone and the taking of the readings. Preliminary tests showed an average difference of only 0.05 between the pH values of aliquot samples of compost tested with the hydrogen electrode and those tested with the quinhydrone electrode.

TESTS FOR CARBON DIOXIDE AND OXYGEN

Samples of air were taken at first from typical mushroom compost heaps at the Arlington Experiment Farm, Rosslyn, Va., 3 or 4 feet high and without artificial ventilation. Later, aspirations were made from commercial storage heaps 5 to 6 feet high, and from heaps with artificial ventilation at the ground level. In all heaps without artificial ventilation the oxygen content of the air in the interstices of the compost decreased steadily as the bottom center of the heap was approached from the sides or from the top. Anaerobic conditions were usually found within 2 or 3 feet from the side of the heap and 1 foot from the upper surface. Carbon dioxide analyses of the same samples of air indicated a corresponding increase in carbon dioxide as the lower center of the heap was approached. These phenomena are presumably due to the presence of an actively respiring microbial flora.

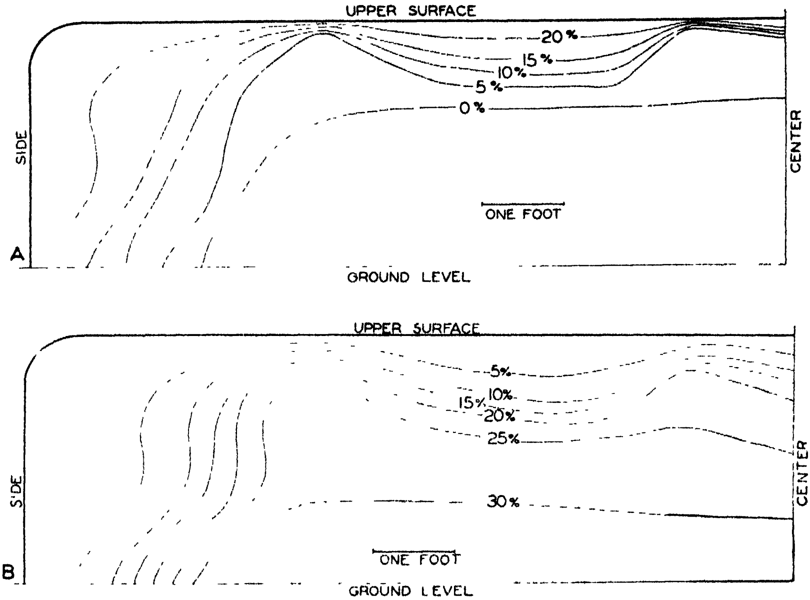

FIGURE 1.—Cross section of a mushroom compost heap from side to center, 12 feet from the end, showing the concentrations of oxygen (*A*) and carbon dioxide (*B*).

Contours showing the concentration of oxygen and carbon dioxide in a typical unventilated heap 3 feet deep are given in figure 1. The dip in the contour lines which appear 5 feet from the side of the heap suggests convection currents.

In most samples in which the oxygen content was more than 1 percent, the sum of the oxygen and carbon dioxide percentages was approximately 21 percent. This, of course, is roughly the percentage of oxygen in the ordinary outside atmosphere, and the constant recurrence of this figure may be taken to indicate that the average respiratory ratio $\left(\frac{ccCO_2}{ccO_2}\right)$ of the heterogeneous microbial population of the compost heap approximates unity under aerobic conditions.

The increase in concentration of carbon dioxide seems to reach a limit at approximately 30 percent. The fact that manure decomposition is arrested in this section is easily observed while the compost heap is being turned, especially during the second turning. At that time the manure in a mound-shaped region in the lower central part of the heap is distinctly "greener" than the remainder of the heap. No attempt was made to determine whether the 30 percent carbon dioxide content is responsible for this retarded fermentation; nor were analyses made of the remaining 70 percent for combustible gases, such as hydrogen and methane, which are known to be generated under similar conditions.

Tests were made to determine the rapidity with which the concentration of carbon dioxide builds up after the manure is turned. As shown in figure 2, it was found that the carbon dioxide content begins to increase rapidly immediately after turning. In 7 hours the carbon dioxide concentration had reached approximately 20 percent, and no oxygen could be detected.

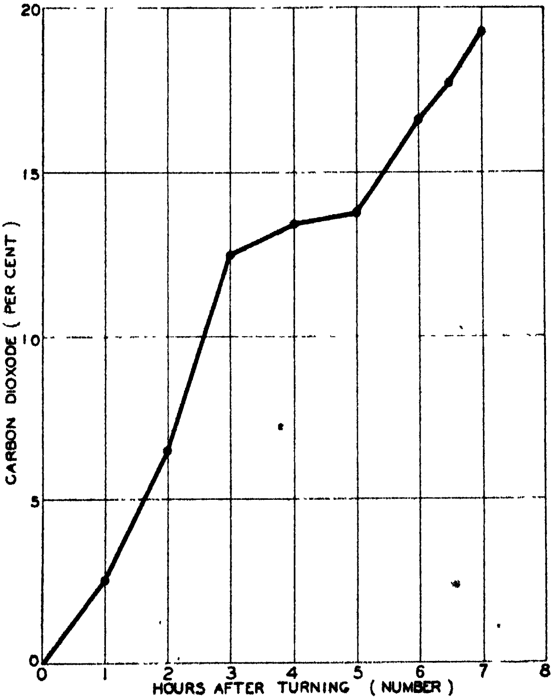

FIGURE 2.—Rate of accumulation of carbon dioxide in center of mushroom compost heap after turning.

For several years a few commercial mushroom growers have been placing ventilating tunnels of lattice work under the center of their compost heaps to speed up decomposition. To determine the effect of ventilation of this kind, bench tile was laid on the ground across an experimental compost heap and aspirations were made at different levels above the tile and at different lateral distances from the tiled area. The results of carbon dioxide analyses from these aspirations are given in figures 3 and 4. It should be noted that there is no anaerobic region in any part of the heap above the ventilation tile. Unlike the conditions in ordinary heaps the percentage of oxygen is higher at the bottom than at 1 or 2 feet from the top. The data in figure 4 indicate that the lateral extension of the effect of ventilation tile on aeration is rather limited. In the heap studied anaerobic conditions prevailed at a lateral distance of 2 feet from the tiled area.

TESTS FOR TEMPERATURE

Two or three days after the compost was mixed the contours of temperatures were found to be substantially the same in all the flat heaps studied. Typical contours are shown in figures 5 to 8. In general the exterior 3 or 4 inches of the compost heaps varies from slightly above air temperature to above 100° F., depending on the moisture content and the tightness with which the manure is packed. Consistent temperatures begin to be found at a depth of about 6 inches. At the ground level temperatures are relatively low, usually from 110° to 120° at points within the heap 2 to 4 feet from the side, then dropping as the center of the heap is approached until temperatures of less than 100° may be encountered. Above the ground there is a similar temperature range. The low-temperature region forms a low mound in the center of the pile roughly corresponding to the anaerobic region. Above this the layers of successively higher temperatures rise in more or less regular strata, following the outlines of the mound. The hottest portion of the heap occupies the space between the sides of the heap and the central anaerobic mound. It usually extends from 6 inches to 2 feet down from the top and from 1 to 4 feet in from the sides. Though usually somewhat oval, it

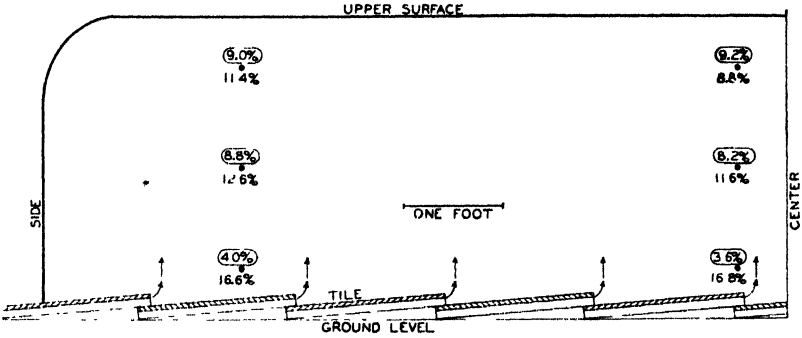

FIGURE 3.—Cross section of one half of compost heap ventilated with greenhouse bench tiles at ground level. Numbers encircled represent concentrations of carbon dioxide; other numbers represent concentrations of oxygen, at points indicated by dots. Arrows indicate currents of air

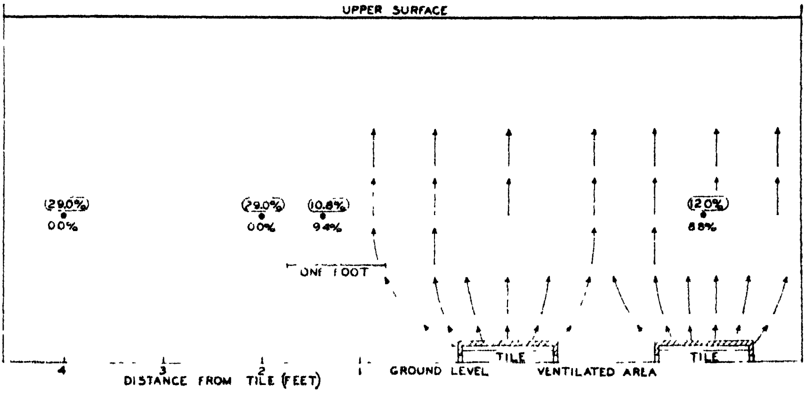

FIGURE 4.—Longitudinal section of portion of compost heap ventilated with greenhouse bench tiles. Section taken across tiles to show percentages of oxygen and carbon dioxide immediately above, and at different lateral distances from source of air. Numbers encircled represent percentages of carbon dioxide; other numbers represent percentages of oxygen, taken at points indicated by dots. Arrows represent currents of air.

varies in shape and may contain from 2 to 5 square feet in cross section. In this region, forming a ring like a huge elongated doughnut about the center of the pile, the temperature is usually in the neighborhood of 170°, although 182° was recorded on one occasion.

In view of the work of numerous investigators on microbial thermogenesis (*6*, *7*, *8*),[2] there can be little doubt that this distribution

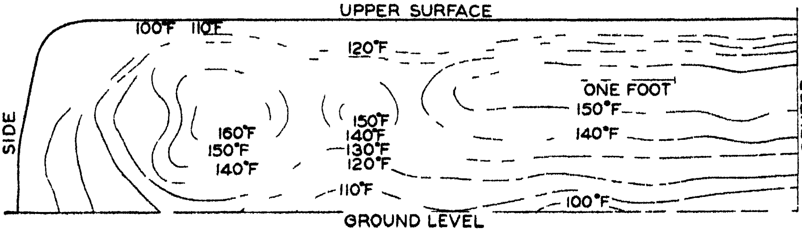

FIGURE 5.—Cross section 6 feet from end of compost heap 2 feet high, showing temperature contours

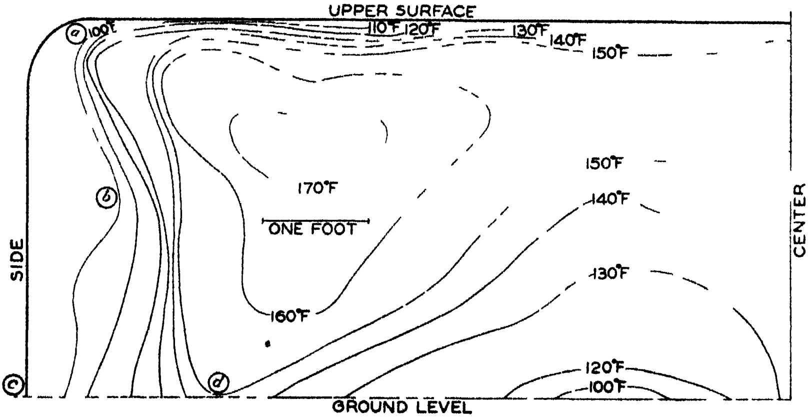

FIGURE 6.—Cross section through compost heap 4 feet high, showing temperature contours. Letters in circles designate points referred to in the text.

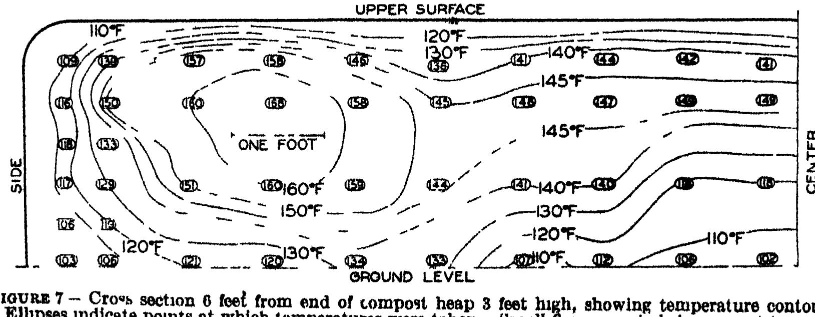

FIGURE 7.—Cross section 6 feet from end of compost heap 3 feet high, showing temperature contours. Ellipses indicate points at which temperatures were taken. Small figures encircled represent temperatures at those points.

of temperature is due largely to the thermogenic activities of an active microbial population.

The warm region apparently is favorable for the accumulation of heat because it is well insulated from outside temperatures and at the same time is comparatively well supplied with oxygen. The outer layers are cooler because of the lack of insulation from the outside; and the lower central region is cooler because the lack of oxygen retards the microbial activity. The slight extension of the high-temperature region into the adjacent anaerobic region is probably due to the conduction of heat from the aerobic region.

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[2] Reference is made by number (italic) to Literature Cited, pp. 600, 601.

Although moderate aeration seems to be necessary for the production of high temperatures, an excessive current of air through decomposing material may have a cooling effect. This was especially noticeable in heaps of artificial manure made at the Arlington Experiment Farm in the fall of 1930. In these experiments some heaps were made with straw as it came from the straw stack; whereas other heaps were made with the same weight of straw and chemicals, but the straw used had been chopped by means of a corn cutter into 3-inch lengths. There were 12 pairs of these comparable heaps, and in every case during the early part of the composting period the heaps made with short straw were from 40° to 80° F. warmer than those made with long straw. After a few weeks of composting, when the long straw had lost much of its stiffness, the difference was not so noticeable.

Irregularities in the exterior contours of the heaps illustrated in figures 5 and 6 may also have been caused by excessive aeration due to convection currents. In hand-turned piles there is usually more or less "flaking", or stratification, on oblique lines from the center, and the air passes into the heap along these strata. This is probably the cause of the convex contour in figure 6, the warm air rising at *a*, drawing in cool air at *c*, and causing the convexity at *b*. The extension of the hotter regions toward *d* is probably due to oxygen brought in by this fresh air, which is available for the heating of the manure beyond the point where the cooling effect of the excessive aeration is felt. In more uniformly turned piles the contours would be expected to be more regular, as in figure 7.

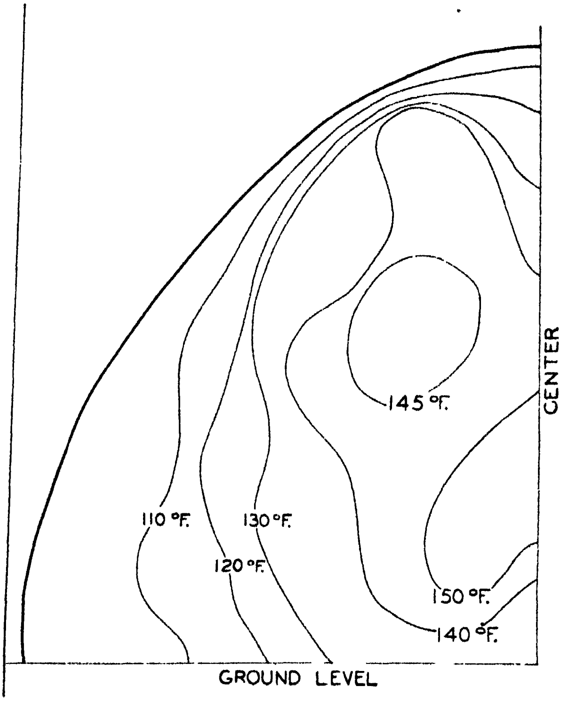

FIGURE 8.— One half of cross section through center of conical compost heap, showing temperature contours.

In heaps of the same area, but 2 feet in depth, the contour pattern is practically the same on a vertically compressed scale as in the higher heaps, except that the cool central mound tends to occupy a slightly greater proportion of the heap than is the case in the higher heaps (fig. 6).

A fairly typical contour pattern for conical heaps is shown in figure 8. In this type of heap the cool central region at ground level is relatively small, but the cooling effect of outside air being drawn in at the lower sides influences a relatively far greater region.

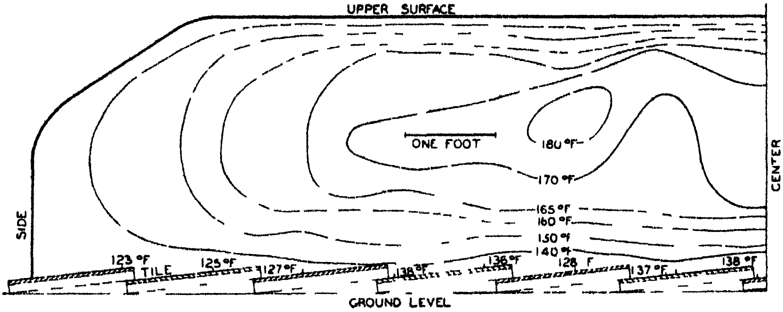

FIGURE 9.—Cross section of compost heap ventilated with greenhouse bench tiles, taken parallel to tiles. Note that whole bottom layer is above 120° F.

In large heaps of the "ridged" type, used in some places, the cross section resembles that of the conical heap, except that the sides are perpendicular to a height of 3 or 4 feet and taper thence to a truncate ridge. These piles are 50 to 60 feet long. In these heaps it would be expected that the contours in cross section would resemble those in figure 8 more or less closely. Because the currents of air have access on only two sides, it is probable that the cooler region at the lower sides will be smaller, and that in the center at ground level larger.

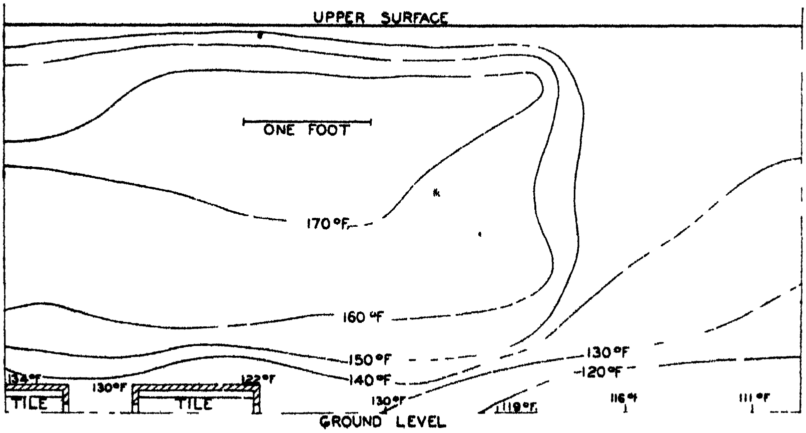

FIGURE 10.—Longitudinal section of compost heap ventilated with greenhouse bench tiles, taken at right angles to tiles.

As pointed out in the discussion of aeration, for some time it has been the practice of a few growers to place beneath the composting heaps heavy lattice troughs, or other means of admitting air to the bottom. The effect of this procedure on the temperature contours is shown in figures 9 and 10. It will be seen that the temperature at the ground level is raised uniformly to above 120° F. In the upper strata the high-temperature areas coalesce, so that the temperature contours are flattened out and the temperature of the whole heap is raised and made more nearly uniform. Undoubtedly this is due to the comparatively uniform distribution of oxygen that was present over the ventilation tiles. When temperatures were taken in a plane at right angles to the tiles, it was found that the parts of the heap more than a foot away from the tiles at ground level were not much affected. As would be expected, a foot or so above the tile the heat of the manure extends laterally for a somewhat greater distance. From the data at hand, however, it would seem that the lateral extension of the effect of aerating devices is rather limited.

In order to ascertain how long it would take compost to attain its maximum heat after turning, with its attendant aeration, the bulbs of recording thermometers were placed in various portions of the 4-foot heaps. In the heaps without tile the temperatures usually reached their maximum in from 18 to 24 hours in the portions of the pile well off the ground but at points at or near ground level continued climbing for 144 to 168 hours or more, as shown in figure 11. In these piles the difference in temperature between the lowest and highest points at the end of a week or 10 days may be as much as 40° F. and was usually in the neighborhood of 20°. By this time the temperatures are usually running along fairly evenly or dropping slightly. The influence of external weather conditions is very evident. There is a sharp rise in temperature, even at the bottom of the pile, at about noon on warm days, and a corresponding drop at night. Rain causes a sharp drop and a nearly equally sharp rise in temperature, affecting the upper portions of the heaps more than those lower down.

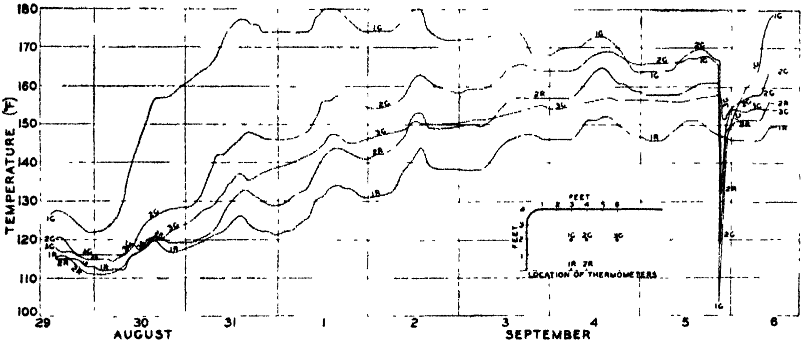

FIGURE 11.—Record of temperatures in different parts of mushroom compost heap after the second turning. The small diagram indicates the positions of recording thermometers by dots and by the symbols 1-G, 2-G, 1-R, 2-R, etc.

In ventilated compost heaps the temperatures rise more slowly, attaining their maximums in from 48 to 72 hours, the lower portion of the heap being the slowest to warm up. After the maximum temperature has been reached and held for 24 hours or so the temperature slowly drops until the next turning, descending perhaps 25°, as shown in figure 12. The difference between points in the lower and upper portions of the heaps is, as has been pointed out, much less, being only about 8°. The influence of external weather conditions is seen in these piles but is very much less evident than in the unventilated ones.

HYDROGEN-ION CONCENTRATIONS

There is a considerable difference in the opinion of different workers on the question of the acidity of the compost. Duggar (*4*) in 1905 stated that manure which has undergone fermentation for a few weeks is usually slightly acid in reaction. This statement was accepted for 20 years and substantiated by Bechman (*2*), who found a reaction of pH 6.4 in manure that had fermented 21 days. On the other hand, Beach (*1*) and Lambert (*9*) found an alkaline reaction in numerous samples of mushroom compost from commercial establishments in eastern Pennsylvania. The pH values recorded in the present study indicate an alkaline condition in well-aerated compost and an acid condition in the parts of the heap composting under anaerobic conditions. It is possible that the discrepancies in the results of different workers can be explained on this basis.

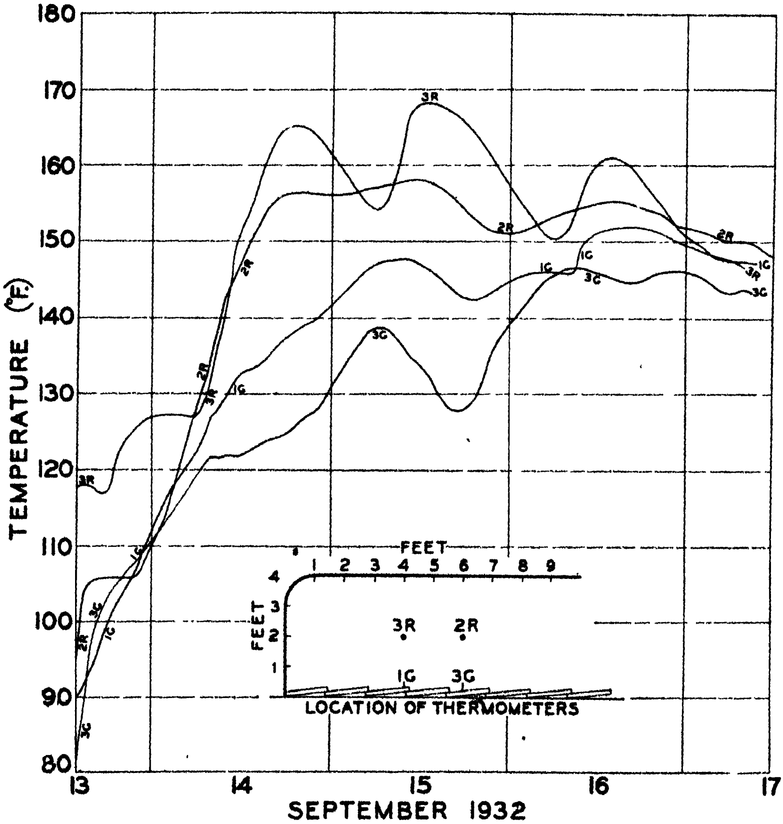

FIGURE 12.—Record of temperatures over tiled portion of mushroom compost heap. The small diagram indicates positions of recording thermometers by dots and by the symbols, 1-G, 3-G, 2-R, and 3-R.

The results of a series of tests of the pH value in different parts of a compost heap are given in figure 13. It is apparent that the outside layer of this heap was largely alkaline or neutral (pH 8.5 to 7.1), whereas the anaerobic mound at the bottom of the heap was predominantly slightly acid (pH 6.6 to 5.1). The general trend from an alkaline reaction in the outside layers toward a slightly acid reaction in the lower central portion is unmistakable, although there are several notable exceptions. Manure subject to firefang was neutral or alkaline in reaction, and manure over tile ventilation as a rule was more alkaline than manure taken from the bottom of unventilated heaps.

MOISTURE CONTENT

Moisture is one of the most variable factors in a compost heap. In general, mushroom growers attempt to maintain approximately 150 percent of water in the compost on a dry-weight basis. Water is usually added during the process of turning the compost, and in many cases soil is added to the manure to help conserve the moisture. As a result of these practices a moderate moisture content is maintained in most of the compost. On the other hand, there is always a tendency for the compost to dry out excessively on the sides of the heap. This is undoubtedly due to the taking up of moisture by currents of air which are warmed upon entering the heap. A tendency to dry out is noticeable also in the layer of manure in contact with ground-level ventilators. A converse condition is noticeable in the upper 6 inches of the heap. Here the warm air from the lower regions rises to the surface saturated with moisture that condenses when the air reaches the cool outer shell. As a result there is usually a wet layer over the surface of the heap. Toward the end of the composting period there is usually less tendency for the compost to dry out than in the beginning when the straw in the manure is still stiff.

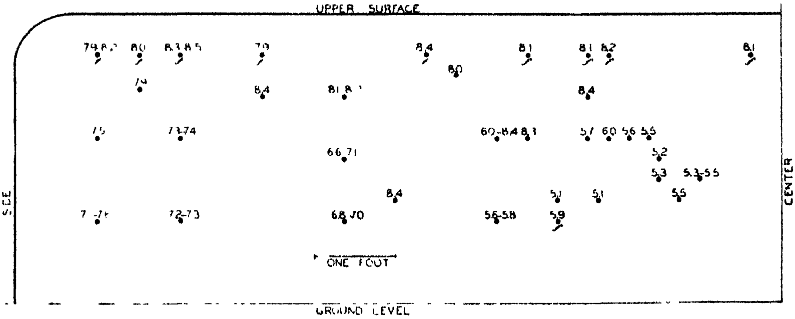

FIGURE 13.—Cross section of compost heap, showing pH values of composting manure, 7 days after second turning, taken at points indicated by dots The surface layer is alkaline (pH 8 0 to 8.5). The aerated layers on the sides of the heap range from pH 7.1 to 7 6. The wet layer on the bottom center is usually acid (pH 5 1 to 6.6), with occasional alkaline spots (pH 8 0 to 8 4). The firefanged manure (*f*) is aklaline as a rule.

DISCUSSION AND CONCLUSIONS

It is evident from the data presented that there are markedly different conditions of aeration, temperature, acidity, moisture, and rate of decomposition in different parts of ordinary flat mushroom compost heaps and that these conditions are distributed in a regular manner that is fairly consistent for heaps of similar size and shape. Perhaps it would be well to point out here the changes in these conditions to be expected from changes in the size and shape of the heap and some of the implications of these phenomena in the general problem of improving the composting practice for mushroom culture.

Considering aeration first, it has been shown that there is a progressive reduction in oxygen accompanied by an increase in carbon dioxide in the interstices of the manure as the center of a compost heap is approached from the outside, and that 8 hours after turning, anaerobic conditions prevail in regions deeper than 1 foot from the top of the heap and more than 3 feet within the side of the heap. Likewise, it is apparent that when ventilators are run under the center of the heap along the ground the anaerobic condition, high in carbon dioxide, in the lower central part of the heap, is changed to a fairly well aerated one. Vertically this change extends from the ventilators to the top of the heap, but laterally the aeration does not seem to extend more than 2 feet. It is evident from these observations that increases in the height or the width of unventilated compost heaps tend to increase the proportion of manure subject to anaerobic conditions over that subject to aerobic conditions. On the other band, anaerobic conditions can be entirely eliminated by the use of closely spaced ground ventilators.

The distribution of temperature in the compost heap seems to be dependent on three factors, namely, aeration, conduction, and convection. The highest temperatures (160° to 180° F.) are usually confined to a region 2 to 4 feet within the sides of the heap and 1 to 3 feet from the top. The outer layers are cooler because of the lack of insulation from the outside and the lower central region is cooler because the lack of oxygen retards microbial thermogenesis. Since thermogenesis is retarded by a lack of oxygen, changes in the height or width of the heap can be expected to affect the average temperature in much the same way as aeration is changed. Increases in the height or width of the heap reduce the average temperature by increasing the size of the cool central region, and complementary ventilators placed at the ground level materially raise the average temperature of the heap.

A region containing compost having an acid reaction and having a comparatively slow rate of decomposition corresponds roughly with an anaerobic region, and the proportion of compost subject to these conditions is increased also with increases in the height and width of the heap.

It is a common observation that currents of air passing into the sides of compost heaps or through ventilators at the ground level have a tendency to dry out the compost at the sides of the heap and surrounding the ventilators. Therefore, reducing the width of a heap increases the tendency for it to dry out during composting, and the insertion of ventilators at the ground level has a similar tendency.

It would seem then that within reasonable limits decreasing the lateral dimensions of a compost heap, or reducing the width of the heap as compared to the length, tends to increase the proportion of aerated alkaline compost in the heap, to raise the average temperature, and to increase the tendency of the compost to dry out between turnings. The insertion of ventilators at the ground level has a similar effect. On the other hand, increasing the height of the compost heap, as is frequently done when manure is stored for several weeks, tends to increase the proportion of anaerobic acid compost in the heap, to decrease the average temperature, and to some extent to reduce the tendency toward drying out.

The foregoing considerations naturally raise the question, What composting conditions are likely to produce the most favorable medium for the growth and yield of mushrooms? At the present time this question cannot be answered in a categorical fashion in terms of size, shape, ventilation, and methods of turning the compost heap. Most commercial growers, when using manure of average texture, make up their heaps about 4 feet high, 20 feet wide, and 40 to 60 feet long. In these heaps about one half of the manure composts under anaerobic conditions; and if it were not for the thorough mixing obtained during the turning process, the lower central part of the heap would take 2 or 3 times as long to decompose as the outer portion. Preliminary experiments and the beneficial effect of the final fermentation in the beds suggest that an aerated condition in the compost heap is preferable to an anaerobic condition provided it can be attained without excessive heating or drying out. Theoretically aeration can be increased by making the heaps narrower or lower, by inserting ventilators at the ground level, or, perhaps preferably, by both increasing the height of the heap and inserting ground-level ventilators. Such changes seem worthy of experimental trial, but it should be recalled that they also may tend toward excessive drying out and overheating and that the beneficial effects of aeration are not well established. The problem can probably best be attacked by a series of semi-empirical yield experiments combined with a study of the microbial and insect population encouraged under different conditions. The large number of factors to be considered and the heterogeneity of stable manure, composting conditions, and conditions during the growth of the crop will make sure progress slow and expensive.

As a working hypothesis it may be assumed that composting conditions which produce a favorable medium for the development of mushrooms probably do so because they encourage the development of a microbial population that is best able to pave the way for the subsequent growth and fructification of mushrooms. Such a hypothesis must take full cognizance of the effect of the staling products of different groups of organisms on mushroom development as well as the action of these organisms in producing changes in the manure favorable to the nutrition of mushrooms under competitive conditions. Interesting facts pertinent to the latter question have been brought to light by the culture studies of Styer (*10*, *11*) and Bechman (*2*) and the proximate chemical analyses of Hébert (*5*) and of Waksman and Niessen (*12*).

Raising the temperatures approximately 25° F. at the bottom of the heap by ground-level ventilation suggests interesting possibilities from the standpoint of reducing the introduction of pests into the mushroom houses with the composted manure. If all houses could be properly heated, fumigated during the peak of the heat, and protected thereafter, there would be much less trouble from insect and fungus pests; but at present this ideal is usually not attained, and it is important that the compost be taken into the house as nearly pest-free as possible. In the unventilated heaps a considerable portion of the bottom layer is below 100°, and a still greater proportion below

110°, temperatures that most mushroom pests can survive for some time. It is true that the high carbon dioxide concentration and the lack of oxygen might cause insect and fungus pests to cease activity, but they probably can survive for a long time under those conditions. Temperatures necessary to kill mushroom insects of various species in their various stages have not yet been determined with accuracy but are certainly below 130°. Chapman (*3*) gives 125.6° as the highest authentic record of temperature endured by any insect. In the case of fungus pests the benefits are more uncertain, because some fungus pests are known to withstand temperatures higher than 130°.

SUMMARY

Gas samples taken from all parts of mushroom compost heaps indicate an increase of carbon dioxide and decrease of oxygen toward the lower central part of the heap. In flat heaps 3 feet deep anaerobic conditions are usually found deeper than 1 foot and more than 3 feet from the sides of the heap. Compost in this portion of the heap tends to be acid, while that in well-aerated portions is alkaline or neutral. The highest temperatures (160° to 180° F.) are usually confined to a region 2 to 4 feet from the sides of the heap and 1 to 3 feet from the top. The outer layers are cooler because of the lack of insulation from the outside and the lower central region is cooler because the lack of oxygen retards the microbial activity. At ground level temperatures (100° to 120°) are usually lower than in the higher strata, presumably also because of lack of oxygen. A more uniform distribution of oxygen and wider distribution of the high-temperature region is induced by placing ventilating tiles at ground level. In all probability, conditions such as these influence the suitability of the finished compost for mushroom culture by establishing the trend of the microbial and insect population of the compost heap.

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IRREGULARITIES IN THE INHERITANCE OF THE HAIRY-NECK CHARACTER TRANSPOSED FROM SECALE TO TRITICUM[1]

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INTRODUCTION

A preliminary paper[2] recorded the transfer of the "hairy neck" of rye (*Secale*) to wheat (*Triticum*). This transfer of a definite character is of interest inasmuch as within *Secale* there are certain economic characters, particularly winter hardiness, that are desired in the common wheats. If it can be shown that one character may be successfully transferred from one genus to the other there are good reasons to believe that other characters also may be transferred. This paper deals primarily with the genetic stability and with the behavior of these hairy-neck wheat forms in crosses with different varieties of wheat.

REVIEW OF LITERATURE

Leighty and Taylor[2] and Florell[3] report the isolation of hairy-neck lines from wheat-rye hybrids. Bleier[4] gives a comprehensive review of the work of investigators who have studied phases of the wheat-rye problem. Florell[3] has reviewed studies on the cytology of wheat-rye hybrids.

MATERIALS AND METHODS

Hairy-neck is characterized by the presence of pubescence or hairiness on the peduncle, or that portion of the culm just below the first node of the rachis (fig. 1). In rye plants and in hairy-neck wheatlike segregates from wheat-rye crosses hairiness varies from a few hairs around the apical node of the culm to a dense pubescence extending 3 or more inches below the head.

As reported by Leighty and Taylor,[5] typical wheatlike hairy-neck segregates were selected in 1923 at the Arlington Experiment Farm, near Washington, D.C., from descendants of natural wheat-rye hybrids found in 1918. Ten of these selections were grown originally, but later work was concentrated on three, designated as C, K, and H, which it is believed represent the characteristic behavior of this group of selections.

The three selections are similar to *Triticum vulgare*[6] in their principal spike characters, with the exception of the neck (or peduncle), which is hairy, as shown in figure 2. The plants are not so tall and

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[1] Received for publication Nov. 18, 1933; issued June 1934

[2] LEIGHTY, C. E., and TAYLOR, J. W. "HAIRY NECK" WHEAT SEGREGATES FROM WHEAT-RYE HYBRIDS. Jour. Agr. Research 28: 567–576, illus 1924.

[3] FLORELL, V. H. A CYTOLOGICAL STUDY OF WHEAT-RYE HYBRIDS AND BACK CROSSES. Jour. Agr Research 42: 341–362, illus. 1931.

[4] BLEIER, H. GENETIK UND CYTOLOGIE TEILWEISE UND GANZ STERILER GETREIDEBASTARDEN. Bibliog. Genetica 4: 321–400, illus. 1928.

[5] LEIGHTY, C. E., and TAYLOR, J. W. See footnote 2.

[6] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum*, but as *T. vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writer gives preference to that form.

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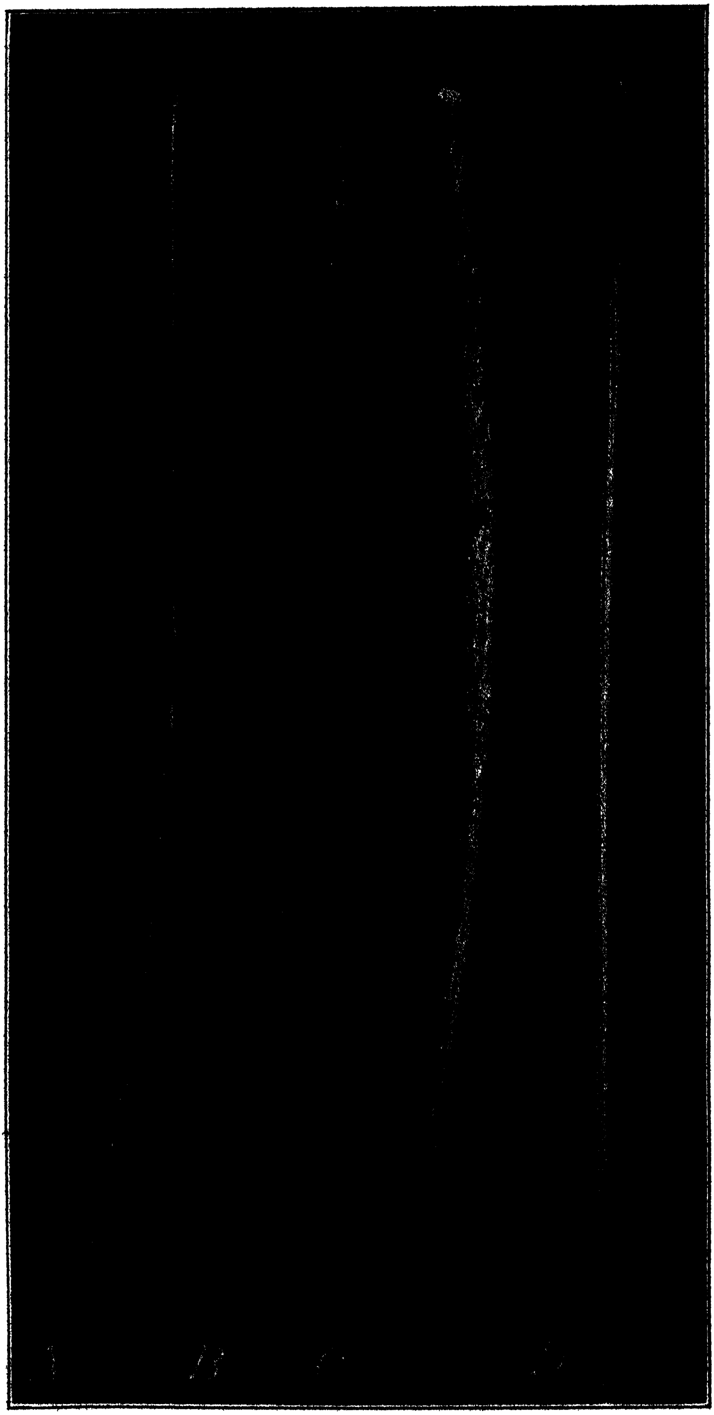

FIGURE 1.—Necks (upper portions of peduncle) of rye, wheat, and two hairy-neck wheatlike selections from wheat-rye hybrids. *A*, Abruzzes rye; *B*, Selection C; *C*, Selection H; *D*, Fulcaster wheat. × 3. *A*, *B*, and *C* are hairy.

are less vigorous than those of wheat, as is usually the case with hairy-neck segregates of wheat-rye crosses. They are more subject to natural crossing than are commercial varieties of wheat, and selfing has been necessary to maintain them. They may be described as follows:

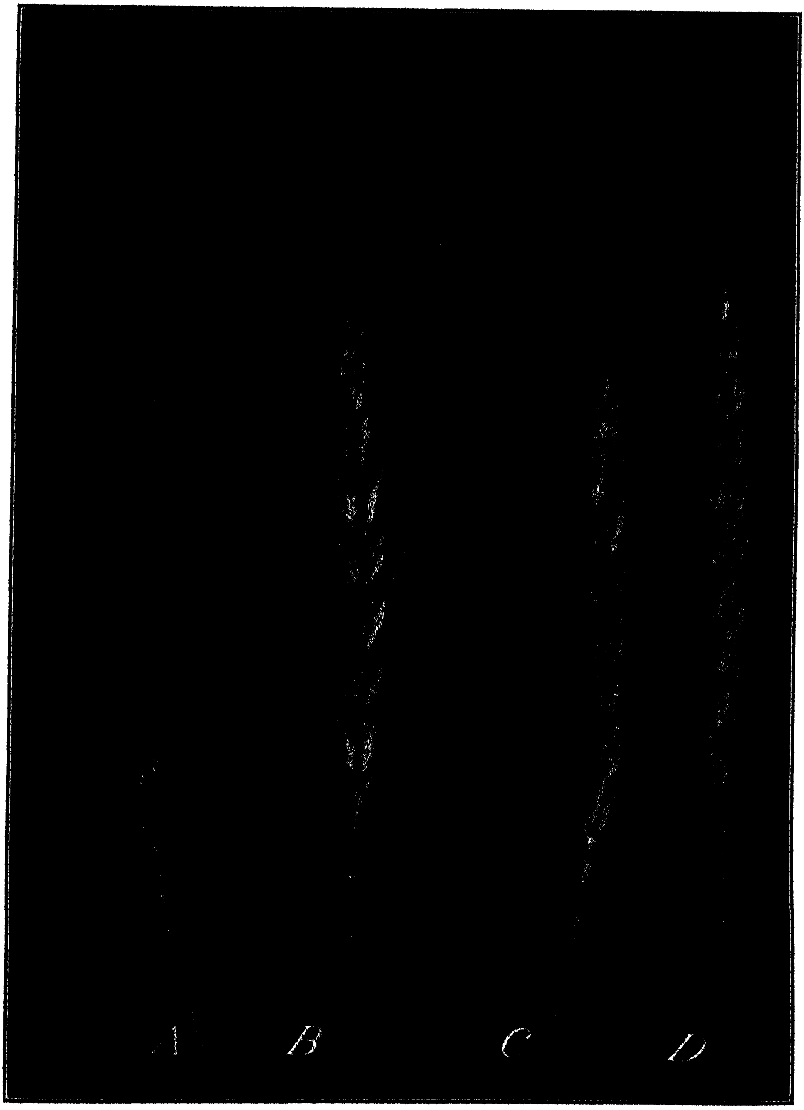

FIGURE 2.—Heads of wheat and three hairy-neck wheatlike selections from wheat-rye hybrids. *A*, Selection K; *B*, Selection H; *C*, Fulcaster wheat; *D*, Selection C. Natural size.

SELECTION C.—Awnless, white glumes, red kernel, with hairs extending one half inch downward from the apical node.

SELECTION K.—Awned, white glumes, red kernel, with hairs extending one half inch downward from the apical node.

SELECTION H.—Awned, white glumes, red kernel, with hairs extending 4 inches or more downward from the apical node. Spike more lax than that of Selection K.

Observations regarding the stability of each of the three selections with respect to the hairy-neck character were made. Each of these selections was crossed with several varieties of soft red winter wheat grown at the Arlington Farm and the progeny were studied in such a way as to determine the nature of the segregation of the hairy-neck character in relation to other characters of the parents. The F_2 populations were grown in spaced nursery rows and the F_3 populations in 5-foot head rows. All hairy-neck plants of each F_2 population which produced sufficient seed for a test were continued in the F_3 generation. Notes on neck character and height were taken in the field. Data on sterility were obtained from the two lower florets of each spikelet of the primary head.

EXPERIMENTAL RESULTS

CONSTANCY OF THE HAIRY-NECK SELECTIONS

The constancy of the hairy-neck character was determined by selfing hairy-neck plants of each of the selections and recording the number of aberrant types appearing in the following generation. The data for Selection C, which is regarded as typical of the hairy-neck segregates, and which has been selfed since 1925, are given in table 1. It will be seen that Selection C does not stand the test of genetic purity expected of a true species. Of 3,818 plants grown during the 8 years, 262, or nearly 7 percent, were different from those of Selection C with respect to the hairy neck.

TABLE 1.—*Constancy of the hairy-neck character in progeny of Selection C selfed for 8 generations*

Year	Total plants	Plants similar to Selection C	Plants differing from Selection C	
			Variant hairy	Variant smooth
	Number	*Percent*	*Percent*	*Percent*
1924	39	92.3	7.7	0.0
1925	675	93.8	5.9	.3
1926	608	94.7	4.8	.5
1927	388	99.0	1.0	0
1928	435	93.5	5.1	1.4
1929	227	97.8	1.8	.4
1930	423	91.0	5.9	3.1
1931	1,023	89.2	9.6	1.2
Total or average	3,818	93.1	5.9	1.0

Two aberrant types appeared, one, designated "variant smooth", indistinguishable from wheat, and the other, designated "variant hairy", almost intermediate with respect to hairy neck between Selection C and wheat. There were approximately 1 percent of the former and 6 percent of the latter. Variant smooth is easily distinguished from Selection C because it is smooth-necked and taller. Variant hairy is from 4 to 6 inches taller than Selection C when grown under favorable conditions, but the difference in height may not be apparent under unfavorable conditions. Partly for this reason it is not so readily recognized. However, there is good reason to believe that none of the conclusions arrived at herein is invalidated by errors of classification.

During the 8 years Selection C never behaved as a pure line. Variant hairy necks were found every year and variant smooth necks in 6 of the 8 years. The greatest irregularity occurred in 1931, when 110 plants of a population of 1,023 plants, or 10.8 percent, were variants. The least variation occurred in 1927, when only 1 percent were variants.

Additional data regarding the constancy of Selection C and the breeding behavior of the variants selected from it were obtained by growing in 1930 a selfed plant of Selection C and selfing the progeny and growing them in 1931. The pertinent data are presented in table 2.

The progeny of the single selfed plant in 1930 were classified as 50 similar to Selection C, 1 variant hairy, and 2 variant smooth. Only 49 of the 50 plants of Selection C indicated in table 2 were grown in 1931, 1 failing to produce sufficient seed. Each of the 49 plant rows supported the 1930 classifications, breeding typical Selection C with 7.3 percent variants. The 2 variant smooth-neck plants bred smooth, and the variant hairy-neck plant segregated in the ratio of 3 smooth to 1 hairy.

TABLE 2.—*Breeding behavior of the hairy-neck character in the progeny of a plant of Selection C during 2 generations of selfing, 1930 and 1931*

Progeny from selfed plant, 1930		Progeny from second generation of selfing, 1931			
			Type of plant		
Type	Total plants	Total plants	Selection C	Variant hairy	Variant smooth
	Number	*Number*	*Percent*	*Percent*	*Percent*
tion C	50	384	92.7	7.0	0.3
ant hairy	1	8	0	25.0	75.0
ant smooth	2	26	.0	.0	100.0

The percentage of smooth-neck plants in this particular line of Selection C in 1930 and 1931 was somewhat less (0.7 percent), and the proportion of variant hairy plants slightly more (6.4 percent) than the average shown in table 1.

During this study of the inconstancy of Selection C 30 variant hairy-neck plants were grown in head or plant rows. These produced 1,388 plants of which 321, or 23.1 percent, were hairy-necked and 76.9 percent smooth-necked, thus approximating the results, presented later, of crosses between Selection C and wheat.

The average proportion of smooth-neck plants appearing in Selection C, that is, about 1 percent, may be explained by assuming a simultaneous loss of the hairy-neck factor in 10 percent of the pollen cells and egg cells. The expected proportion of smooth-neck, variant hairy-neck (heterozygous), and Selection C types is then given by the expansion of the binomial $(1+9)^2$. The fact that the smooth-neck plants bred true and the hairy-neck plants bred like F_1 hybrids is in accord with this hypothesis. However, the average proportion of variant hairy-neck plants, approximately 6 percent, is only about one third of the number to be expected on this basis. It seems necessary to assume also differential functioning or vigor of the two types of gamet or zygotes, or it is possible that the loss of the hairy-neck factor may occur in a somatic division in the development of the primordium for the flowers of a spike.

INHERITANCE IN CROSSES OF HAIRY-NECK SELECTIONS × WHEAT

Hybridizing the hairy-neck selections with common wheat may be expected to give further information as to the genetic irregularity of the hairy-neck character and its relation to the inheritance of certain common wheat characters. In 1923 and later, selections which from phenotypic indications were pure for the hairy-neck character of the C, K, and H selections, were crossed with common wheat varieties. The varieties chosen differed in such head characters as awnlessness and awnedness, red and white glume color, and smoothness and pubescence of glumes. The segregation of the common allelomorphs permitted observation as to the effect of an intergeneric character on their behavior.

The F_1 hybrids conformed in the more common spike characters to what would be expected in crosses of wheat varieties; that is, there was expressed the incomplete dominance of awnlessness, red glume color, and pubescent chaff. The hairy-neck character was dominant, but the hairs did not extend downward so far as in the parental selection, and the density of the hairiness was decidedly reduced. The F_1 heads appeared fully fertile and were selfed.

The hairy-neck selections were crossed with one or more of the varieties of *Triticum vulgare*, namely, Brown Fife,[7] Purplestraw, Fulcaster, Nittany, Poole, and Fultz. All except Fulcaster and Nittany are awnless, and all except Brown Fife and Poole have glabrous white glumes. Brown Fife has pubescent red glumes, and Poole has glabrous red glumes.

Glume color developed poorly, and segregates from this character were not classified, although it was evident that hairy neck was present in both the red- and white-glume segregates of the F_2 generation. The number of F_1 plants secured in each case, the number of F_2 plants that were grown, and the segregation of the latter with respect to presence of awns, pubescence of glumes, and hairy necks are shown in table 3.

The segregation with respect to awns and pubescence is what would be expected when varieties of *Triticum vulgare* possessing these characters are crossed. In the six crosses involving awn segregation, the fully awned recessive constitutes 24.4 percent of the total number of plants that were grown. In the single cross (Selection C × Brown Fife) involving pubescent and glabrous glumes, 24.1 percent of the plants had glabrous glumes. On the other hand, the segregation with respect to the hairy-neck character was quite irregular. In the five crosses involving Selection C the percentage of hairy-neck plants ranged from 17.7 to 29.2 and averaged 25. In the four crosses involving Selection K, the percentage of hairy-neck plants ranged from 30.6 to 48.2, with an average of 36.2. There were two crosses involving Selection H. In these the percentages of hairy-neck plants were 61 and 63.4, respectively, averaging 62.9.

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[7] The name "Brown Fife" was given in 1922 to a strain of wheat formerly grown as Jones Winter Fife. In habit of growth and morphological characters it is somewhat similar to Grandprize.

Table 3.—*Segregation in the F_2 generation from crosses of 3 hairy-neck selections with varieties of common wheat at the Arlington Experiment Farm, Rosslyn, Va.*

Cross	F_1 plants	F_2 plants	F_2 plants of indicated class					
			Awnless				Awned (glabrous)	
			Pubescent		Glabrous			
			Hairy	Smooth	Hairy	Smooth	Hairy	Smooth
	Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Selection C × Brown Fife	1	220	12.3	63.6	5.5	18.6	0.0	0.0
Selection C × Purplestraw	2	1,365	.0	.0	26.4	73.6	.0	.0
Selection C × Fulcaster	1	168	.0	.0	22.6	57.7	6.6	13.1
Nittany × Selection C	12	343	.0	.0	15.2	58.6	5.8	20.4
Selection C × Poole	1	171	.0	.0	26.9	73.1	.0	.0
Selection K × Purplestraw	4	486	0	0	37.7	39.5	10.5	12.3
Selection K × Fultz	13	281	.0	.0	23.8	50.2	8.2	17.8
Fulcaster × Selection K	1	191	0	.0	.0	.0	38.7	61.3
Poole × Selection K	8	950	0	0	21.9	52.1	8.7	17.3
Selection H × Fultz	1	100	.0	.0	51.0	27.0	10.0	12.0
Selection H × Fulcaster	9	383	0	.0	0	.0	63.4	36.6

In none of the crosses involving Selections K and H do the ratios conform to simple Mendelian inheritance. The average results for Selection C agree exactly with expectations for a monohybrid, except that hairy neck behaves as the recessive, whereas in the F_1 this character was dominant. The breeding behavior of the F_1 of Selection C × wheat is similar to that of the hairy-neck variants.

There is no indication of linkage of the hairy-neck character with either of the other characters studied except in the Selection H × Fultz cross, in which the proportion of hairy necks in a small population is approximately twice as great for the awnless segregates as for the awned.

A number of the crosses were continued into the F_3 generation. Some of these were space-planted, but the greater number were grown in 5-foot head rows. In some cases all the plants from the F_2 rows were grown, whereas in others only the hairy-neck plants were grown. The progeny of 388 smooth-neck F_2 plants were grown and all bred smooth neck. The data for the hairy-neck plants are presented in table 4.

Of the 125 F_3 lines grown from hairy-neck F_2 plants of the two crosses of Selection C, only 3, or 2.4 percent, were homozygous. If the hairy-neck character were a simple recessive, 33.3 percent should be homozygous.

Of the 83 hairy-neck F_2 plants of the cross Selection K × Fultz grown in the F_3, approximately 11 percent were homozygous hairy neck. However, of the 76 F_3 lines of the cross Selection K × Purplestraw 25 percent were homozygous. In the F_2 of Selection K × Purplestraw approximately 50 percent of the plants were hairy neck as compared to 32 percent in the cross Selection K × Fultz. In the former cross the F_2 homozygous hairy-neck plants appeared more than twice as often as in the latter cross.

TABLE 4.—*Breeding behavior of hairy-neck F_2 plants from crosses of hairy-neck selections × wheat*

Cross	F_3 lines		
	Number	Heterozygous hairy neck	Homozygous hairy neck
		Percent	*Percent*
Selection C × Purplestraw	71	98.6	1.4
Nittany × Selection C	54	96.3	3.7
Selection K × Fultz	83	89.2	10.8
Selection K × Purplestraw	76	75.0	25.0
Selection H × Fultz	54	81.5	18.5

The F_1 of Selection K × Fultz was grown in 1925 and the F_1 of Selection K × Purplestraw in 1928. The difference in percentage of homozygosity of the two crosses is believed to be due to differences in the two seasons, inasmuch as 30 of the segregating F_3 lines of Selection K × Purplestraw, involving 545 plants, were space-planted and 25.5 percent of the plants were hairy as compared to approximately 48.1 percent in the F_2 generation. The higher percentage of hairy-neck plants in the F_2 generation of this cross as compared to that of the other three crosses of Selection K × wheat, and the comparatively high percentage of F_3 homozygous hairy-neck lines, indicate conditions unusually favorable for either the formation or the functioning or both of the hairy-neck gametes of the F_1 plants.

Of the 54 F_3 lines of Selection H × Fultz, 18.5 percent were homozygous hairy-neck. Selection H crosses produced approximately 61 percent of hairy-neck plants in the F_2; that is, the hairy-neck character behaved as dominant. However, the F_3 test clearly shows a lethal factor operating to eliminate the homozygous hairy-neck type.

EFFECT OF HAIRY NECK ON PLANT CHARACTERS

The supposition of a lethal effect of the hairy-neck factor suggested the desirability of a study of sterility, seed germination, height of plant, and tillering of the crosses, especially with respect to the smooth-neck and hairy-neck segregates.

STERILITY

The percentages of sterile florets of the hairy-neck selections and of the F_1 hybrids between these and certain varieties of wheat are shown in table 5.

TABLE 5.—*Floret sterility of hairy-neck selections and of F_1 hybrids of hairy-neck selections × wheat*

Selection or F_1 hybrid	Florets	Seeds	Sterile florets
	Number	*Number*	*Percent*
Selection C	1,190	789	33.7
Nittany × Selection C	369	321	13.0
Selection C × Purplestraw	1,356	1,249	7.9
Selection K	282	159	43.6
Selection K × Fultz	536	487	9.1
Selection H	204	160	21.6
Selection H × Fultz	106	102	3.8

In Selection C, 33.7 percent of the florets were sterile, and in the F_1 hybrid of Selection C × wheat and its reciprocal, approximately 10 percent of the florets were sterile. This is about the average sterility for wheat. The average sterility of Selection K was 43.6 percent and of Selection H, 21.6 percent. The F_1 hybrids with wheat in each case were as fertile as would be expected for wheat, the sterility of Selection H × wheat being only 3.8 percent. Selection H has the highest fertility of the three selections and the same relation exists between the F_1 hybrids with wheat. It is pertinent in this connection to note that in the F_2 generation 61 percent of the plants of this cross had hairy necks as compared with 25 and 36 percent in Selections C and K, respectively (table 3). In Selection H, hairiness extends 4 inches down the peduncle as compared to approximately one half inch in the other two selections; that is, the degree of hairiness in these selections was not positively correlated with reduction of fertility as might be expected.

SEED GERMINATION

Seed of the F_1 plants of wheat × Selection C was planted and allowed to mature. Approximately 85 percent of the planted seeds matured plants. A similar study was made of Selection K × wheat from F_2 seed, as no F_1 seed was available. Conditions for germination were poor, and only 49 percent of the seed of segregating hairy-neck lines and a like percentage of homozygous hairy-neck lines matured plants. A similar planting of smooth-neck seed from the same cross matured only 48 percent of plants. In neither case is there any evidence of differential zygotic lethals.

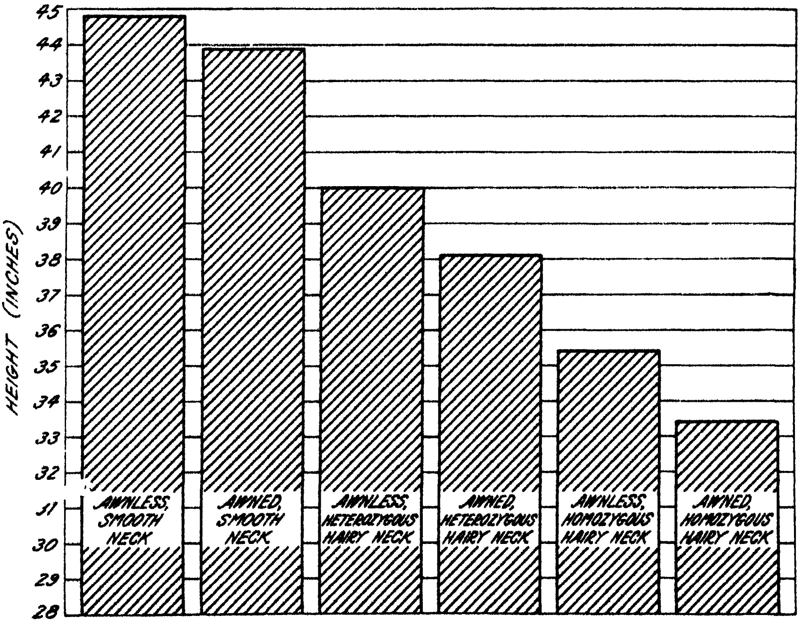

FIGURE 3.—Height of plants of different awn and neck types in the F_3 generation of the cross Selection K × wheat.

HEIGHT OF PLANT

The height of a large number of plants in the F_2 and F_3 generations was measured, and the data are presented in table 6. In each of the 17 possible comparisons of the hairy-neck with the smooth-neck classes the hairy-neck plants were significantly shorter, in most cases materially so. The average height of the smooth-neck plants was 46.9 inches as compared to 41.4 inches for the hairy-neck plants. Furthermore, the homozygous hairy-neck F_3 lines were approximately from 2 to 5 inches shorter than those segregating for hairy neck. The comparative height differences in the classes obtained from the cross of Selection K × Fultz are shown graphically in figure 3. No significant differences were found between the height of the plants as a result of the presence or absence of awns.

The commonly cultivated rye varieties have hairy necks. A few strains of smooth-neck rye have been bred, the height of which is no greater than that of their hairy-neck relatives. It is probable, therefore, that the hairy-neck character in the presence of the rye-chromosome set does not adversely affect the height of the plant. The average height of the rye varieties grown at the Arlington Experiment Farm varies from 62 to 65 inches as compared with 46 and 54 inches in the wheat varieties.

TABLE 6.—*Average height of hairy-neck and smooth-neck plants in heterozygous and homozygous hairy-neck lines from hybrids of hairy-neck selections × wheat*

Class and hybrid	Generation	Plants	Average height of plants of indicated class			
			Awnless smooth	Awnless hairy	Awned smooth	Awned hairy
HETEROZYGOUS		*Number*	*Inches*	*Inches*	*Inches*	*Inches*
Selection K × Fultz	F_2	281	47.5±0.42	40.7±0.39	47.7±0.40	38.7±0.85
Do	F_3	1,466	44.8± .36	40.0± .38	43 9± .47	38.1± .57
Selection C × Purplestraw	F_2	1,172	44.1± .39	39.6± .45		
Selection H × Fultz	F_2	90	47.5± .55	42.8± .66	47.6± .69	40.9±1.83
Do	F_3	443	49.1± .14	45.2± .16	49 8± .11	46.9± .16
HOMOZYGOUS LINES						
Awnless hairy (Selection C × Purplestraw)	F_3			36.1± .44		
Awned hairy (Selection K × Fultz)	F_3					33.4± .80
Awnless hairy (Selection K × Fultz)	F_3			35.4± .97		
Awned hairy (Selection H × Fultz)	F_3					44.8± .80
Awnless hairy (Selection H × Fultz)	F_3			43 2± .72		

TILLERING

Data on tillering were obtained for individual plants in the F_2 generation of the two crosses Selection K × Fultz and Selection H × Fultz. The former was grown on more productive land than the latter. In all cases the smooth-neck plants tillered more than did the hairy-neck plants (table 7). The differences between awned and awnless plants were not statistically significant.

Table 7.—*Average number of tillers per plant in F_2 classes of hairy-neck selections × wheat crosses*

Cross	Average tillers per plant in indicated F_2 class					
	Awnless			Awned		
	Hairy	Smooth	Difference	Hairy	Smooth	Difference
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Selection K × Fultz	6.7±0.39	8.5±0.46	1.8±0.60	6.6±0.58	9.5±0.46	2.9±0.74
Selection H × Fultz	3.7± .12	5.3± .30	1.6± .32	3.4± .37	4.5± .49	1.1± .61

BACK-CROSSING F_1 HYBRIDS WITH WHEAT

Since no evidence of zygotic elimination was obtained it seemed desirable to resort to back-crossing to test for comparative functioning of male and female gametes carrying the smooth-neck and hairy-neck factors. This was done by reciprocally crossing the F_1 hybrids with wheat, only the F_1 hybrids of Selection C and Selection K being used. The resulting progeny were then classified with respect to the hairy-neck character. Errors due to self-pollinated plants were eliminated by the selection of a wheat variety in which selfing could be detected. The data are presented in table 8.

The female gametes of Selection K × Fultz, fertilized by wheat pollen, produced plants of which 16.9 percent had hairy necks, whereas the male gametes of the same hybrid, fertilizing wheat egg cells, produced but 9.0 percent of hairy-neck plants. Similarly, the female gametes of the F_1 of Selection C × wheat (Purplestraw and Nittany), fertilized by wheat pollen, produced 13.2 percent of hairy-neck plants, whereas the male gametes of the same hybrid, fertilizing wheat egg cells, produced 8.9 percent of hairy-neck plants. In all back crosses except one a larger percentage of hairy-neck plants was produced when the F_1 hybrid was used as the female parent. However, neither the functional male nor female gametes carried the hairy-neck character in the expected frequency, since in back-crossing experiments such as these the hairy-neck and smooth-neck gametes should occur in equal numbers.

Table 8.—*Hairy-neck and smooth-neck plants resulting from reciprocal back-crossing of the F_1 hybrid of hairy-neck selections × wheat with wheat*

F_1 hybrid	Year	F_1 hybrid as the female produced—			F_1 hybrid as the male produced—		
		Smooth-neck plants	Hairy-neck plants		Smooth-neck plants	Hairy-neck plants	
		Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Selection K × Fultz	1925	23	6	20.7	17	3	15.0
Do	1926	46	8	14.8	54	4	6.9
Total or percent		69	14	16.9	71	7	9.0
Selection C × Purplestraw	1926	34	6	15.0	46	3	6.1
Nittany × Selection C	1929	18	5	21.7	6	0	0
Selection C × Purplestraw	1930	93	11	10.6	92	11	10.7
Total or percent		145	22	13.2	144	14	8.9

Theoretically, there should be agreement among the F_2 segregation, the F_3 family behavior (that is, whether homozygous or heterozygous for the neck character), and the results from the back crosses. The latter indicated that approximately 16.9 percent of the functional eggs and 9.0 percent of the male cells of Selection K × Fultz (table 8) carry the hairy-neck factor. Assuming the same gametic frequency (1+5) (1+10) and the same functioning in the selfed F_1 hairy neck × wheat hybrids, the F_2 population should contain 24.2 percent of hairy-neck plants, and approximately 6.3 percent of these should be homozygous in F_3. Actually, 32 percent of the F_2 plants were hairy (table 3), and 10.8 percent of the F_3 lines were homozygous (table 4).

In Selection C × wheat slightly more than 13 percent of the egg cells and about 9 percent of the male cells carried the hairy-neck character. On this basis the F_2 population from the selfed F_1 hybrids should be approximately 21.0 percent hairy neck, and 5.7 percent of these should be homozygous. The percentage of the hairy-neck plants actually observed in the F_2 generation of the same crosses (Purplestraw and Nittany) was 25.3 (table 3), and 2.4 percent of these bred true (table 4).

The agreement between the data of the different experiments is perhaps as close as should be expected, considering the small populations obtained from the back crosses and the apparent irregularity in genetic expression due to environment.

DIFFERENTIAL FUNCTIONING OF POLLEN CELLS

The low percentage of functional gametes carrying the hairy-neck factor, as shown in the reciprocal back crosses of the F_1 hybrids of hairy-neck selections × wheat with wheat, would indicate elimination of the hairy-neck character at meiosis or at fertilization. The high percentage of fertility in the F_1 hybrids of hairy-neck selections × wheat favors the assumption that functional female gametes carrying the hairy-neck factor are not formed in the expected frequency. The apparent differential functioning of the male gamete of the F_1 hybrids carrying the hairy-neck and smooth-neck characters (table 8) may, however, be due to a growth differential between the two types rather than to nonformation of pollen cells carrying the hairy-neck factor. Experiments were therefore made with mixtures of pollen of Selection C and pollen of three varieties of common wheat, namely, Nittany, Dixie, and Red Rock. Heads of the wheat or of Selection C were emasculated and at the proper time were pollinated with a pollen mixture or first with pollen of Selection C and then with pollen of the wheat variety; in the latter case the interval between the two pollinations averaged about 2 minutes. The pollen mixture was composed of the contents of the same number of anthers of Selection C and of the wheat variety. The anthers of Selection C are larger than those of the wheat varieties used.

When wheat was the female, the progenies were grown and classified as smooth neck or hairy neck, the results showing which pollen grain functioned. When Selection C was the female, glume color or awn contrast of the following progenies showed when the wheat pollen grain functioned, except in Dixie, when the plants were carried to the F_2 generation to identify them. Results of the pollinations are shown [illegible] table 9.

Table 9.—*Comparative functioning of pollen of hairy-neck selection C and wheat varieties in pollen-mixture and double-pollination experiments*

Female parent	Year	Pollen source	Plants of indicated type resulting from pollination			Flowers fertilized by pollen carrying hairy neck
			Wheat	Selection C	Hybrid	
			Number	*Number*	*Number*	*Percent*
Nittany	1928	Mixture Nittany and Selection C	10		1	
Dixie		Mixture Dixie and Selection C	11		0	
Red Rock	1929	Mixture Red Rock and Selection C	17		2	
Nittany	1928	Selection C and Nittany	11		3	
Dixie		Selection C and Dixie	12		1	
Red Rock	1929	Selection C and Red Rock	42		5	
Total			103		12	10.4
Selection C	1928	Mixture Dixie and Selection C		2	22	
Do		Selection C and Nittany		2	14	
Do		Selection C and Dixie		0	7	
Do	1929	Selection C and Red Rock		1	8	
Total				5	51	8.9

One hundred and fifteen plants resulted from pollinating the common wheat varieties with pollen from the two sources. Only 12, or 10.4 percent, were hairy-neck hybrids, the remainder being selfs. When the wheats were pollinated with the mixture the percentage of hybrids was 7.3, and when pollinated first with pollen of Selection C and then selfed the percentage of hairy-neck hybrids increased to 12.2, possibly indicating an effect due to rate of pollen germination or of pollen-tube growth.

Fifty-six plants were secured in the experiments in which Selection C was the female. Fifty-one, or 91 percent, proved to be hybrids and only 5, or 9 percent, were selfs. Approximately the same number of flowers of wheat and of Selection C were pollinated in these experiments, and the fewer seeds and plants obtained indicates again the sterility of Selection C as compared with that of wheat. These results suggest that the pollen cells of Selection C which carry the hairy-neck factor are less viable or that the pollen tube grows more slowly than that of normal wheat. Poor functioning of pollen cells carrying the hairy-neck factor appears at least as probable as nonformation at meiosis in the F_1 hybrid. This is further supported by the agreement between the results from back-crossing the F_1 of Selection C × wheat with wheat (table 8) and the results from pollinating wheat and Selection C with the pollen mixture. Wheat fertilized with pollen from the F_1 hybrid (Selection C × wheat) produced 8.9 percent of hairy-neck plants, whereas wheat fertilized with a mixture of pollen from Selection C and wheat produced 10.4 percent of hairy-neck plants; and Selection C fertilized by a mixture of pollen from Selection C and wheat produced 8.9 percent of selfed hairy-neck plants (table 9).

DISCUSSION

The genetic behavior of the hairy-neck wheatlike selections isolated from wheat-rye hybrids shows that the addition of the rye character results in an unbalanced type. Hairy-neck is a tangible rye character transposed to types that are apparently otherwise *Triticum vulgare*. A preliminary cytological examination of one of the hairy-neck plants made by Florell[8] showed 44 chromosomes in the root tips as compared to 42 for *T. vulgare*. Inasmuch as the hairy-neck plants are not constant, their chromosomal constitution seems to be better represented by the quantitative expression $2n+2$ rather than $2n$, indicating in this case no homologue in the wheat complement for the rye chromosome. Blakeslee[9] uses the formula $2n+2$ for one of his Globe mutants in *Datura* where the unbalance was of a simple tetrasomic type.

To explain the genetic behavior of the hairy-neck character it may be assumed that the $2n+2$ hairy-neck plants normally produce $n+1$ gametes but that occasionally in male and female gametogenesis the rye chromosome is lost, giving a gamete of n constitution. The fertilization of $n+1\times n$ gametes results in a zygote similar in later behavior to the cross hairy-neck selection × wheat, whereas the mating of $n\times n$ gives a zygote which produces a plant indistinguishable from *Triticum vulgare*.

The chromosome number of the F_1 hybrid hairy-neck selection × *Triticum vulgare*, and also of the variant hairy type, would be $2n+1$ and the plants would be of the hairy-neck type as the character is dominant over the smooth neck. In gametogenesis and fertilization, irregularities in the functioning of $n+1$ and n gametes apparently occur, as the F_2 segregation often shows the hairy character as recessive, and results from the back crosses indicate that from 13 to 17 percent of the functional egg cells and approximately 9 percent of the functional pollen grains carry the hairy character. Furthermore, F_3 lines homozygous for hairy neck do not appear in the expected frequency even for a recessive character. Reduced height and tillering and varying degrees of sterility in the plants with hairy neck as compared to those with smooth neck, in addition to the genetic irregularities, support the belief that there is incompatibility between the wheat and rye complexes and that the reaction is unfavorable both to the normal productiveness of the plant and to its constancy in breeding. Whether the addition or substitution of other rye chromosomes in the wheat complement would react similarly is questionable. Wheat-rye hybrids carrying all the chromosomes in both wheat and rye, $2n=56$, have been produced,[10] but the economic value of such plants has not seemed particularly promising in the United States. Wheat breeders in general are interested in obtaining a definitely *T. vulgare* type with certain desired rye characters rather than a type intermediate between the two genera.

SUMMARY AND CONCLUSIONS

Complete genetic balance has not been obtained in three hairy-neck selections of wheat × rye crosses designated as Selection C, Selection K, and Selection H. In spite of continuous selfing, approximately 1 percent of the plants of Selection C had smooth necks and bred true and about 6 percent had hairy necks and bred in the same manner as the F_1 hybrids.

The observed proportion of smooth-neck plants may be explained by assuming a simultaneous loss of the hairy-neck factor in 10 percent of the pollen cells and egg cells, but the observed proportion of hybrid hairy-neck plants has been only about one third of the number to be expected on the basis of this explanation. It seems necessary to assume also differential functioning or vigor of the two types of gametes or of the zygotes, or possibly loss of the hairy-neck character in somatic mitosis.

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[8] Letter addressed to J. W. Taylor by V. H. Florell, Feb. 28, 1931.

[9] BLAKESLEE, A. F. VARIATIONS IN DATURA DUE TO CHROMOSOME NUMBER. Amer. Nat. 56: 16-31, illus. 1922.

[10] LEVITSKY, G. A., and BENETZKAIA, G. K. CYTOLOGICAL INVESTIGATIONS OF CONSTANT INTERMEDIATE RYE-WHEAT HYBRIDS. (PRELIMINARY COMMUNICATION.) U.S.S.R. Cong. Genet., Plant and Animal Breeding, Proc. 2: 345-352, illus. 1930. [In Russian. English Summary, pp. 350-352.]

In crosses between the three selections and several varieties of wheat the hairy-neck character appeared to be dominant, but in later generations it behaved as a recessive or in an irregular manner.

There appeared to be no linkage of the hairy-neck character with glume color, with condition of glumes in regard to pubescence, or with condition of heads in regard to awns.

In studies of sterility it was found that Selection C, Selection H, and Selection K were materially less fertile than wheat, but that the F_1 hybrids were approximately as fertile as wheat. There was no observable inverse relation between degree of hairiness and sterility, as might be expected; Selection H, which had more hair on the necks than the others, was the most fertile.

In a comparison of the germination of segregating hairy-neck lines, homozygous hairy-neck lines, and homozygous smooth-neck lines, no differences were observed.

In study of the height of plants and of vigor as measured by tillering, it was found that in crosses between the three selections and wheat the smooth-neck segregates were invariably taller than the hairy-neck segregates from the same cross. It was also found that heterozygous hairy-neck segregates were taller than homozygous hairy-neck segregates. In all cases smooth-neck plants from these crosses tillered more than comparable hairy-neck plants.

The F_1 hybrids were reciprocally back-crossed with wheat. In all crosses but one, a larger percentage of hairy-neck plants was produced when the F_1 hybrid was used as a female parent. In the one exception there was practically no difference. There was good agreement among the data secured by back-crossing, the F_2 segregation, and the breeding behavior of the F_3 lines.

A study of differential functioning of pollen grains was made by using mixtures of pollen of Selection C and one of three varieties of wheat. The florets were emasculated and either pollinated with a mixture of pollen or pollinated first with pollen from Selection C and about 2 minutes later with pollen from wheat. The results indicated that the pollen cells of Selection C are less viable or that the pollen tube grows more slowly than in wheat. There was apparently no discrimination on the part of the egg toward either type of gamete.

Since the hairy-neck plants are irregular in their breeding behavior, it seems logical to represent their chromosomal constitution by the expression $2n+2$ rather than by $2n$, indicating no homologue in wheat for the rye chromosome carrying the hairy-neck factor. It may then be assumed that the hairy-neck plants produce $n+1$ gametes and that occasionally the rye chromosome is lost, giving a gamete of n consitution. The union of $n+1$ and n gametes results in a zygote similar to that produced by a cross of a hairy-neck selection × wheat, and the union of n gametes produces a plant which cannot be distinguished from wheat.

THE COMPARATIVE EFFECTIVENESS, IN THE DAIRY RATION, OF SUPPLEMENTS OF PHOSPHORUS IN THE FORM OF ORTHOPHOSPHORIC ACID, MONOSODIUM, DISODIUM, TRISODIUM PHOSPHATES, AND BONE MEAL [1]

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INTRODUCTION

Studies of calcium and phosphorus metabolism carried on for a number of years at the United States Dairy Experiment Station at Beltsville, Md., have shown that a proper adjustment of the mineral content of the ration is of vital importance to health, milk production, and reproduction in the dairy cow.

The work of Shohl (*5*)[2] with rats a few years ago suggested the possibility that the assimilation of calcium and phosphorus might be affected by a change in the acid-base ratio of the ration. Shohl found the greatest retention of calcium and phosphorus in rats on a neutral diet. The neutral diet was obtained by adding orthophosphoric acid to an alkaline ration. The alkaline ration alone produced the symptoms of tetany and the acid ration (made by adding phosphoric and hydrochloric acid to the alkaline ration) produced the symptoms of rickets.

One of the writers (*6*) has shown that for favorable assimilation of calcium and phosphorus by dairy cows the calcium-phosphorus ratio should not be too wide. Dairy rations of alfalfa hay and grain often have a rather large proportion of calcium as compared with phosphorus.

The experiments here reported were undertaken (1) to study the effect of variations in the alkalinity of the ration on the calcium and phosphorus metabolism in cows, and (2) to determine the form in which supplements of phosphorus could best be supplied. In the first experiment the ration was supplemented with soluble phosphates; in the second experiment bone meal was used. An attempt was made to eliminate the effect of any organic food constituents by feeding a uniformly good quality of hay and grain throughout.

FIRST EXPERIMENT: ORTHOPHOSPHORIC ACID, MONOSODIUM, DISODIUM, AND TRISODIUM PHOSPHATES AS SUPPLEMENTS OF PHOSPHORUS

In the first experiment a basal ration somewhat low in phosphorus was used and phosphorus supplements were added in the form of orthophosphoric acid, monosodium, disodium, and trisodium phosphates. These were added in such amounts as to maintain a calcium-

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[1] Received for publication Nov. 23, 1933; issued June, 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 630.

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phosphorus ratio in the feed below 1.50, preferably about 1.25. Under such conditions it was hoped that some superiority of one form of supplement over another would be apparent.

Calculated, according to Shohl, on the basis of inorganic acids and bases in the ration, 100 grams of this basal ration was equivalent to 63.3 cubic centimeters normal alkali. The amount of phosphoric acid added was in no case sufficient to neutralize the alkalinity. This method of calculating the reaction of the ration, however, disregards carbonates and organic acids. It may be said to give a rough idea of the titratable alkalinity of the ration, but it gives no idea of what the hydrogen-ion concentration would be in the kind of watery extract that is formed when such a ration is introduced into the alimentary tract. The amounts of orthophosphoric acid and of trisodium phosphate used in this experiment may well have been sufficient to produce definite changes in the hydrogen-ion concentration of the alimentary contents in the early stages of digestion.

The provision for additional phosphorus, at least in the form of the more neutral supplements, has proved valuable and the results have led to certain conclusions which will be discussed later.

EXPERIMENTAL PROCEDURE

For this experiment three Holstein cows were used. Cow 265 was a purebred, and cows A–37 and A–40 were grades. Cows A–37 and A–40 were about 4 years old, and cow 265 was 9 years old. All three cows were pregnant and in the fourth month of lactation when the experiment was started, but cows A–37 and 265 aborted early in the experiment after about 2 months of pregnancy. They were bred again and, at the end of the experiment, cow A–37 had completed 5 months of pregnancy; cow A–40, 7 months; and cow 265, 1.5 months.

The experiment began September 20 and ended March 13, a period of 25 weeks. During the first 4 weeks a basal ration was fed, consisting of U.S. No. 1 grade alfalfa hay and a grain mixture (whole yellow corn meal, 40 parts; wheat bran, 30 parts; soybean meal, 20 parts; linseed meal, 10 parts; and sodium chloride, 1 part). The cows were given as much feed as they would "clean up" and an effort was made to maintain about equal consumption of grain and hay.

Because of the hot weather at this time (early fall) it was difficult to induce the animals to consume sufficient feed to meet their energy requirements, particularly in the case of cow 265. This cow was offered a little timothy hay. She seemed to relish it, and since it had been observed in other experiments at this station (*2*) that animals at times indicated a preference for timothy hay after prolonged periods of alfalfa-hay feeding, it was decided to give all the cows a feeding period on mixed timothy and alfalfa hay. Accordingly, for the next 4 weeks, half of the alfalfa hay of the basal ration was replaced by U.S. No. 1 grade timothy hay. During the following 2 weeks the basal ration was again fed.

During the last 15 weeks of the experiment different phosphorus supplements (equivalent to about 25 to 27 grams of phosphorus daily) were added to the basal ration for periods of 3 weeks each, to learn the effect of varying the alkalinity of the ration.

Beginning with the ninth week, the cows were exercised 10 minutes daily until the end of the experiment. At about that time their appetites began to improve and subsequently their rate of food consumption became steadier. This may be attributed to a combination of factors—exercise, cooler weather, and possibly the feeding of phosphorus supplements.

The weights of the cows at the beginning and end of the experiment were, respectively: Cow A–37, 561 and 651 kilograms; cow A–40, 504 and 577 kilograms; cow 265, 634 and 604 kilograms. The loss of weight by cow 265 was due to the fact that she would not eat sufficient feed to meet her energy requirements.

Hay and grain were fed twice a day and the cows were milked three times a day. Detailed analyses of the feeds are omitted. The alfalfa hay contained approximately 1.5 percent calcium, 0.2 percent phosphorus, and 2.4 percent nitrogen. The timothy hay contained about 0.35 percent calcium, 0.12 percent phosphorus, and 0.82 percent nitrogen. The average grain mixture contained about 0.13 percent calcium, 0.7 percent phosphorus, and 3.4 percent nitrogen. The phosphorus content of the grain was increased by the addition of phosphorus supplements, and was then between 0.9 and 1 percent.

Chemically pure materials were used as supplements and were mixed with the grain. Three parts of sirupy orthophosphoric acid were diluted with 2 parts of water and dropped on the grain as fed each day. During the last 3 weeks of the experiment the phosphoric acid was thoroughly kneaded into the grain mixture to insure actual consumption of the acid.

EXPERIMENTAL RESULTS

Table 1 shows the average weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for each cow during the different feeding periods. The figures for assimilated calcium and phosphorus were calculated as described in a previous publication (*3*).

Both calcium and phosphorus values indicate that the different feeds affected the composition of the milk, probably through changes in the composition of the blood. The phosphorus content of the milk shows a fairly definite tendency to be a little higher during the periods in which the phosphate supplements were fed than at other times. The calcium in the milk is noticeably higher when the cows were on the basal ration than when grain, alfalfa, and timothy were fed, and also shows a tendency to be higher on the orthophosphoric acid supplement than on trisodium phosphate. In the case of cow A–40 this latter tendency is partly masked by the general tendency for the milk calcium to increase during the latter part of lactation, due perhaps to the decreasing milk yield.

The graphs in figure 1 show the fluctuation in the calcium and phosphorus content of the body which occurred during the course of the experiment. The calcium and phosphorus graphs are drawn on different scales, in the ratio of calcium to phosphorus in bone. A variation of 100 grams of calcium corresponds to a variation of 46 grams of phosphorus. If the calcium and phosphorus balances signify a building up or breaking down of bone material only in the body, then the graphs should follow an identical course. The fact that considerable divergence is shown between the calcium and phosphorus graphs indicates some difference in the storage possibilities of these two elements in the body, or in the intestinal tract.

TABLE 1.—*Average weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for the 3 cows during the different feeding periods*

Ration, length of feeding period, and cow no	Feed consumed		Milk produced			Calcium						Phosphorus						Calcium-phosphorus ratio in feed
	Grain	Hay	Yield	Composition		In urine and feces	In milk	In feed	Balance	Assimilation		In urine and feces	In milk	In feed	Balance	Assimilation		
				Calcium	Phosphorus													
	Kg	*Kg*	*Kg*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	
Basal ration (4 weeks).																		
Cow A-37	66.5	65.3	143.1	0.121	0.111	874.4	172.8	1,063.7	+16.5	189.3	17.8	433.2	159.2	586.3	−6.1	153.1	26.1	1.81
Cow A-40	60.8	51.0	114.5	.125	.107	742.3	142.8	843.2	−41.9	100.9	12.0	433.0	122.5	520.2	−35.3	87.2	16.8	1.62
Cow 265	66.6	50.4	172.5	.104	.098	700.7	179.6	822.4	−57.9	121.7	14.8	429.0	166.0	549.7	−45.3	120.7	21.9	1.50
Alfalfa, timothy, and grain (4 weeks).																		
Cow A-37	70.0	70.0	135.7	.115	.116	595.5	156.7	727.0	−25.2	131.5	18.1	452.4	157.3	575.5	−34.2	123.2	21.4	1.26
Cow A-40	63.0	55.0	122.1	.123	.109	504.2	150.8	587.2	−67.8	83.0	14.1	417.7	133.4	505.5	−45.6	87.8	17.4	1.16
Cow 265	70.0	47.3	151.1	.099	.094	469.5	149.6	533.1	−86.0	63.6	11.9	468.5	142.8	540.0	−71.3	71.4	13.2	.99
Basal ration (2 weeks):																		
Cow A-37	70.0	70.0	130.7	.119	.115	966.3	156.2	1,127.5	+5.0	161.2	14.3	472.7	151.0	626.0	+2.3	153.3	24.5	1.80
Cow A-40	63.0	54.0	117.3	.127	.106	756.3	148.9	880.8	−24.4	124.5	14.1	428.8	124.9	548.4	−5.3	119.6	21.8	1.61
Cow 265	70.0	42.8	143.4	.101	.092	594.2	144.8	697.9	−41.1	103.7	14.9	451.1	132.7	560.8	−23.0	109.8	19.6	1.24
Basal ration, plus orthophosphoric acid (3 weeks):																		
Cow A-37	70.0	63.0	126.2	.120	.118	894.3	151.8	1,018.2	−27.9	123.9	12.2	662.8	148.8	795.3	−16.3	132.6	16.7	1.28
Cow A-40	63.0	56.0	115.9	.126	.110	767.1	145.6	905.6	−7.1	138.5	15.3	612.0	127.1	733.7	−5.4	121.7	16.6	1.23
Cow 265	70.0	49.5	153.9	.105	.100	685.0	161.5	818.9	−27.6	133.9	16.4	641.4	153.7	769.8	−25.3	128.4	16.7	1.06
Basal ration, plus monosodium phosphate (3 weeks):																		
Cow A-37	70.0	63.0	115.4	.118	.118	872.2	136.2	1,016.1	+7.7	143.9	14.2	640.8	135.8	799.7	+23.1	159.0	19.9	1.27
Cow A-40	63.0	56.0	110.2	.136	.110	728.0	150.2	904.2	+26.0	176.2	19.5	580.8	121.6	718.5	+16.1	137.6	19.2	1.26
Cow 265	70.0	63.0	173.5	.106	.099	808.2	183.3	1,016.1	+24.6	207.9	20.5	617.4	171.1	799.7	+11.2	182.3	22.8	1.27
Basal ration, plus disodium phosphate (3 weeks).																		
Cow A-37	61.7	63.0	97.5	.119	.116	861.2	115.6	1,011.6	+34.8	150.4	14.9	566.9	112.7	687.8	+8.2	120.9	17.6	1.47
Cow A-40	60.0	56.0	102.8	.139	.111	730.9	143.3	905.4	+31.2	174.5	19.3	530.3	114.5	658.1	+13.3	127.8	19.4	1.38
Cow 265	68.3	61.5	166.6	.106	.097	779.4	176.4	975.7	−19.9	196.4	20.1	575.5	161.1	735.9	−.7	160.4	21.8	1.33
Basal ration, plus trisodium phosphate (3 weeks).																		
Cow A-37	61.5	57.3	94.4	.114	.115	799.7	107.6	932.7	+25.4	132.9	14.3	561.5	108.8	657.8	−12.5	96.4	14.6	1.42
Cow A-40	53.3	50.3	87.9	.142	.109	643.7	124.7	818.1	+49.7	174.4	21.3	472.8	96.1	571.8	+2.9	99.1	17.3	1.43
Cow 265	70.0	63.0	170.3	.103	.097	848.8	176.0	1,027.7	+2.9	178.9	17.4	586.7	164.6	744.6	−6.7	157.9	21.2	1.38
Basal ration, plus orthophosphoric acid (3 weeks):																		
Cow A-37	63.0	56.0	83.0	.120	.117	773.5	99.3	912.0	+39.2	138.5	15.2	651.2	97.1	718.0	−30.3	66.9	9.3	1.27
Cow A-40	53.3	46.6	65.2	.154	.110	609.4	100.2	761.4	+51.8	152.0	20.0	551.0	71.7	624.1	+1.4	73.1	11.7	1.22
Cow 265	68.3	63.0	160.4	.104	.094	868.5	166.9	1,022.7	−12.7	154.2	15.1	655.9	150.9	764.0	−42.8	108.1	14.1	1.34

While this experiment was undertaken for the purpose of investigating the effect of variations in the alkalinity of the ration on the calcium and phosphorus metabolism of cows, yet, as the experiment progressed, other factors were indicated as of equal, or possibly greater, importance than the reaction of the ration, namely, the quantity and proportion of calcium and phosphorus in the ration.

A basal ration of alfalfa hay and grain probably does not contain the optimum proportion of phosphorus to calcium for high milk production, even though the grain (containing 30 percent wheat bran) has a fairly high phosphorus content. This is indicated by the fact that all the cows showed excessive losses of phosphorus from the body while on this ration during the first period. Insufficient phosphorus intake and rather generous milk flow are probably the factors causing the negative balances at this time. The calcium-phosphorus ratio in this period ranged from 1.5 to 1.8.

In the second period the intake of calcium was greatly reduced by the substitution of timothy hay for half of the alfalfa in the basal ration; while, in the third period, the calcium intake was increased by a return to the basal ration. The calcium and phosphorus balances became more negative in the second period, and less negative in the third period, but it is doubtful whether these changes in the balances have any physiological significance. The matter can be more profitably discussed in connection with some of the results of the second experiment, which will be given later.

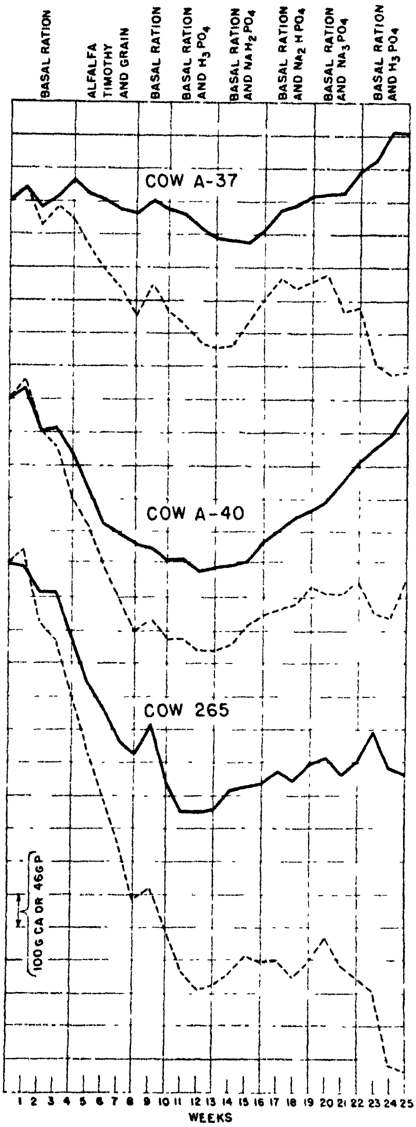

FIGURE 1.—Fluctuation in the content of calcium and phosphorus in the bodies of the cows during the course of the first experiment. The solid line represents calcium; the dotted line, phosphorus. The divisions on the ordinate correspond to 100 grams of calcium or 46 grams of phosphorus, the ratio in which the elements are present in bone.

With the introduction of the phosphorus supplements during the remainder of the experiment the phosphorus intake was materially increased. This increased phosphorus intake did not appear to be very effective in preventing mineral losses, however, when supplied in the form of orthophosphoric acid, as in the fourth and eighth periods. The quantities of phosphorus in the ration and the calcium-phosphorus ratio assumed more appropriate values (average 1.19). In spite of this the animals continued to lose calcium and phosphorus in the fourth period. In the eighth period, when the same supplement was fed, the phosphorus losses were considerable in the case of cows A–37 and 265. Hart (*1*) recently observed, after administering hydrochloric acid to cows, that there was a divergence of the calcium excretion from the feces to the urine but no improvement in calcium balances.

During the fifth and sixth periods, monosodium and disodium phosphates were used as supplements and the effect was marked. Not only were calcium and phosphorus losses checked in the case of all animals but a distinct recovery of mineral stores was initiated which extended even into the seventh period. This occurred without any great reduction in milk yield and while the calcium-phosphorus ratio was being maintained at the above-mentioned appropriate value.

The effect of trisodium phosphate as a supplement was somewhat irregular. Possibly the alkalinity of this material was unfavorable for mineral absorption.

These results would seem to indicate that by merely supplying a suitable neutral phosphorus supplement in sufficient quantity and in proper ratio to the calcium present in the ration (that calcium being already present in an available form and in generous amount), a phosphorus-deficient ration can be made adequate and equilibrium or positive balances can be obtained. It seems reasonable to assume, since the mature high-producing cow's chief need for calcium and phosphorus is to secrete them in the milk, that the ration which she receives should contain calcium and phosphorus in approximately the same proportion that they occur in milk, namely, about 1.1:1. If calcium and phosphorus are supplied in assimilable form and in sufficient quantity and in the proportion present in milk (the ration being satisfactory in other known respects), then, the writers believe, much will have been done to improve the mineral nutrition of the cow.

Phosphorus, in the form of disodium phosphate, was fed to dry cows at this station several years ago (*4*). Very definite increases in milk yield during the subsequent lactations were noted. At that time, however, only a few short-time balances were followed where the cows were receiving a mineral phosphorus supplement.

Since the supplements monosodium and disodium phosphate have brought about so marked a retention of calcium and phosphorus in animals that have suffered considerable mineral losses, they should also be effective in preventing such losses in animals in a better state of nutrition. That such losses, however, are not entirely preventable is evident from an experiment conducted at this station recently (*7*). In this instance two cows giving 21 to 28 kilograms of milk daily were fed the best ration that the writers could devise, including a supplement of disodium phosphate. With average daily calcium and phosphorus intakes of 129 and 117 grams, respectively, these cows were slowly but steadily losing calcium and phosphorus from their bodies. It seems impossible to escape the conclusion recently stated by Hart (*1*), that "in the early period of lactation, *especially with high milk flow*, the calcium assimilation from the digestive tract is insufficient to meet the needs of mammary secretion and the skeleton is drawn upon, with a negative calcium balance resulting." The interdependence of calcium and phosphorus metabolism involves a simultaneously lowered phosphorus assimilation.

SECOND EXPERIMENT: BONE MEAL AS A PHOSPHORUS SUPPLEMENT

A second experiment, conducted in a somewhat different manner, was completed about a year later. In this experiment bone meal was used as a supplement instead of the soluble phosphates used in the first experiment. Bone meal is so often employed as a source of calcium and phosphorus for cattle that it was thought advisable to study its effects on the metabolism of these elements; but, as will appear later, its use introduces experimental complications, which make it necessary to exercise great caution in drawing conclusions as to the physiological significance of the results.

EXPERIMENTAL PROCEDURE

Three grade Holstein cows were used in this experiment—cows A-37, A-43, and A-46. Cows A-37 and A-46 were not pregnant; cow A-43 had been pregnant for about a month at the end of the experiment. The animals were from 3½ to 5 years of age.

The experiment began November 5 and ended January 13, a period of 10 weeks. During the first 3 weeks of the experiment the same basal ration was used as in the first experiment. During the next 7 weeks supplements of bone meal and disodium phosphate were added to the basal ration, bone meal the first 3 weeks and phosphate the last 4 weeks. The amounts used were so regulated as to introduce a uniform increase in the phosphorus intake, that is, one which, when introduced in the form of sodium phosphate, would keep the calcium-phosphorus ratio in the feed between 1.1 and 1.5. Obviously when bone meal was used it was impossible to make this correction in the calcium-phosphorus ratio of the feed. These supplements were added to the grain mixture, and in the case of bone meal, represented about 3.8 percent, and in the case of the disodium phosphate, about 6.5 percent of the mixture.

The weights of the cows at the beginning and end of the experiment were respectively: Cow A-37, 568 and 575 kilograms; cow A-43, 439 and 469 kilograms; cow A-46, 452 and 461 kilograms.

Hay and grain were fed twice a day and the cows were milked three times a day.

The alfalfa hay contained about 1.5 percent calcium and 0.2 percent phosphorus, the grain 0.13 percent calcium and 0.7 percent phosphorus. Chemically pure disodium phosphate and a high grade of bone meal were used to supplement the grain mixture and were thoroughly mixed with it. The bone meal contained 29.45 percent calcium and 14.06 percent phosphorus.

EXPERIMENTAL RESULTS

The milks secreted showed a uniform slight increase in phosphorus content when bone meal was fed (table 2).

Table 2.—*Weekly feed consumption, milk yield, percentage of calcium and phosphorus in the milk, and the calcium and phosphorus balances for the 3 cows during the different feeding periods*

COW A-37

Ration and period	Feed consumed		Milk produced			Calcium						Phosphorus						Calcium-phosphorus ratio in feed*
	Grain	Hay	Yield	Composition		In urine and feces	In milk	In feed	Balance	Assimilation		In urine and feces	In milk	In feed	Balance	Assimilation		
				Calcium	Phosphorus													
	Kg	*Kg*	*Kg*	*Pct.*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Pct.*	
Basal ration:																		
First week	77	77	155.9	0.124	0.119	1,052.3	193.3	1,349.7	+104.1	297.4	22.0	479.7	185.5	679.6	+14.4	199.9	29.4	2.0
Second week	77	77	153.0	.124	.116	1,050.0	189.7	1,352.8	+113.1	302.8	22.4	504.8	177.5	697.9	+15.6	193.1	27.7	2.0
Third week	77	77	149.4	.129	.118	1,130.3	192.7	1,351.9	+28.9	221.6	16.4	542.7	176.3	708.6	−10.4	165.9	23.4	2.0
Average	77	77	152.8	.126	.118	1,077.5	191.9	1,351.5	+82.0	273.9	20.3	509.1	179.8	695.4	+6.5	186.3	26.8	2.0
Basal ration, plus bone meal:																		
Fourth week	77	77	149.5	.120	.121	1,884.1	179.4	2,184.1	+120.6	300.0	13.7	844.6	180.9	1,097.1	+71.6	252.5	23.0	2.0
Fifth week	77	77	142.9	.124	.120	1,880.2	177.2	2,158.1	+100.7	277.9	12.9	857.0	171.5	1,064.0	+35.5	207.0	19.5	2.0
Sixth week	60.5	66	132.4	.119	.119	1,529.7	157.6	1,722.6	+35.3	192.9	11.2	711.5	157.6	845.7	−23.4	134.2	15.9	2.0
Average	71.5	73.3	141.6	.121	.120	1,764.7	171.4	2,021.6	+85.5	256.9	12.6	804.4	170.0	1,002.3	+27.9	197.9	19.5	2.0
Basal ration, plus sodium phosphate:																		
Seventh week	66	77	139.1	.117	.118	1,203.4	162.7	1,262.1	−104.1	58.7	4.7	816.8	164.1	949.4	−31.5	132.6	14.0	1.3
Eighth week	60.5	77	130.6	.117	.114	1,117.9	152.8	1,258.6	−12.1	140.7	11.2	715.4	148.9	868.5	+4.2	153.1	17.6	1.4
Ninth week	55	77	130.3	.119	.118	1,152.5	155.1	1,248.9	−58.7	96.4	7.7	750.8	153.8	838.5	−66.1	87.7	10.5	1.5
Tenth week	66	77	134.7	.119	.117	1,021.2	160.3	1,231.0	+49.5	209.8	17.0	794.3	157.6	944.7	−7.2	150.4	15.9	1.3
Average	61.9	77	133.7	.118	.117	1,123.8	157.7	1,250.2	−31.3	126.4	10.2	769.3	156.1	900.3	−25.2	131.0	14.5	1.4

COW A-43

Ration and period	Grain	Hay	Yield	Calcium	Phosphorus	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Ca assimilation (Grams)	Ca assimilation (Pct.)	P in urine and feces	P in milk	P in feed	P balance	P assimilation (Grams)	P assimilation (Pct.)	Calcium-phosphorus ratio in feed*
Basal ration:																		
First week	70	63	149.0	0.120	0.095	878.4	178.8	1,113.2	+56.0	234.8	21.1	457.4	141.6	603.5	+4.5	146.1	24.2	1.8
Second week	70	63	148.6	.121	.091	869.2	179.8	1,116.0	+67.0	246.8	22.1	463.0	135.2	610.1	+11.9	147.1	24.1	1.8
Third week	70	49.5	139.5	.122	.096	852.1	170.2	895.8	−126.5	43.7	4.9	501.5	133.9	602.2	−33.2	100.7	16.7	1.5
Average	70	58.5	145.7	.121	.094	866.6	176.3	1,041.7	−1.2	175.1	16.0	474.0	136.9	605.3	−5.6	131.3	21.7	1.7

Basal ration, plus bone meal:																		
Fourth week	70	58.5	145.5	.114	.098	1,539.4	165.9	1,823.6	+118.3	284.2	15.6	769.7	142.6	981.8	+69.5	212.1	21.6	1.9
Fifth week	70	63	150.1	.120	.098	1,625.7	180.1	1,854.7	+48.9	229.0	12.3	781.5	147.1	953.1	+24.5	171.6	18.0	1.9
Sixth week	70	63	143.6	.121	.100	1,588.3	173.8	1,787.1	+25.0	198.8	11.1	819.6	143.6	951.7	−11.5	132.1	13.9	1.9
Average	70	61.5	146.4	.118	.099	1,584.5	173.3	1,821.8	+64.1	237.3	13.0	790.3	144.4	962.2	+27.5	171.9	17.8	1.9
Basal ration, plus sodium phosphate:																		
Seventh week	70	63	141.5	.114	.100	940.2	161.3	1,052.7	−48.8	112.5	10.7	868.2	141.5	969.4	−40.3	101.2	10.4	1.1
Eighth week	70	63	146.1	.115	.099	927.9	168.0	1,056.0	−39.9	128.1	12.1	819.5	144.6	955.6	−8.5	136.1	14.2	1.1
Ninth week	70	63	145.3	.117	.098	895.6	170.0	1,052.9	−12.8	157.2	14.9	843.4	142.4	997.9	+12.1	154.5	15.5	1.1
Tenth week	70	63	149.2	.114	.100	907.5	170.1	1,052.8	−50.8	119.3	11.6	833.3	149.2	964.7	−17.8	131.4	13.6	1.1
Average	70	63	145.5	.115	.099	917.8	167.4	1,047.1	−38.1	129.3	12.3	841.1	144.4	971.9	−13.6	130.8	13.4	1.1

COW A-46

Basal ration:																		
First week	70	70	130.6	0.127	0.113	1,002.5	165.9	1,227.0	+58.6	224.5	18.3	477.2	147.6	617.8	−7.0	140.6	22.8	2.0
Second week	70	70	129.9	.124	.109	980.4	161.1	1,229.8	+88.3	249.4	20.3	479.5	141.6	634.4	+13.3	154.9	24.4	1.9
Third week	70	60	120.6	.126	.107	914.4	152.0	1,066.5	+.1	152.1	14.3	499.1	129.0	623.7	−4.4	124.6	20.0	1.7
Average	70	66.6	127.0	.126	.110	965.8	159.7	1,174.4	+49.0	208.7	17.6	485.3	139.4	625.3	+.6	140.0	22.4	1.9
Basal ration, plus bone meal:																		
Fourth week	70	60	124.4	.119	.115	1,589.6	148.0	1,848.5	+110.9	258.9	14.0	794.8	143.1	984.9	+47.0	190.1	19.3	1.9
Fifth week	70	70	126.4	.124	.113	1,715.4	156.7	1,961.9	+89.8	246.5	12.6	796.3	142.8	967.2	+28.1	170.9	17.7	2.0
Sixth week	70	70	122.1	.125	.115	1,721.3	152.6	1,894.3	+20.4	173.0	9.1	838.1	140.4	965.8	−12.7	127.7	13.2	2.0
Average	70	66.6	124.3	.123	.114	1,675.4	152.4	1,901.6	+73.7	226.1	11.9	809.7	142.1	972.6	+20.8	162.9	16.7	2.0
Basal ration, plus sodium phosphate:																		
Seventh week	65	70	117.6	.123	.116	1,071.1	144.6	1,153.6	−62.1	82.5	7.2	801.1	136.4	923.3	−14.2	122.2	13.2	1.2
Eighth week	65	70	116.7	.121	.114	1,016.5	141.2	1,157.0	−.7	140.5	12.1	814.0	133.0	909.1	−37.9	95.1	10.5	1.3
Ninth week	65	70	106.4	.122	.109	1,031.8	129.8	1,154.0	−7.6	122.2	10.6	776.1	116.0	948.1	+56.0	172.0	18.1	1.2
Tenth week	60	70	113.7	.120	.113	1,001.1	136.4	1,119.2	−18.3	118.1	10.6	798.2	128.5	855.2	−71.5	57.0	6.7	1.3
Average	63.7	70	113.6	.122	.113	1,030.1	138.0	1,146.0	−22.2	115.8	10.1	797.4	128.5	908.9	−16.9	111.6	12.1	1.3

Figure 2 shows the fluctuation in calcium and phosphorus content in the bodies of the cows during the course of the experiment.

From a study of table 2 and figure 2 it is evident that the cows retained calcium during the period in which they were on the basal ration (except cow A–43) and that they retained even more calcium as well as phosphorus during the bone-meal supplement period but lost both calcium and phosphorus during the sodium-phosphate supplement period.

The effects of changes in rations on calcium and phosphorus balances noted here are typical of those observed in all balance experiments where the intakes of calcium and phosphorus are varied markedly during the experiment. It is probable, however, that these changes do not represent alterations in the amounts of calcium and phosphorus stored in the bones or other internal tissues of the animal, but changes primarily in the amounts of calcium and phosphorus held in mechanical suspension in the fluid contents of the stomachs and intestines.

The bovine intestinal tract has a very complicated form, consisting of the four stomachs, one of which has highly corrugated walls, in addition to the large intestine and the very long and convoluted small intestine. There can be no doubt that considerable proportions of such insoluble material as tricalcium phosphate, or of any calcium and phosphorus compounds capable of forming tricalcium phosphate, when introduced into this tract with the food, are likely to remain in it for long periods. Furthermore, the normal amount of the bovine intestinal contents or "fill" is very large. In the case of three cows of the Jersey type recently slaughtered at this station after they had fasted for 24 hours, the average amount of fill was 52 kilograms. It is probable that in the case of Holstein cows on full feed the fill would be about 100 kilograms.

Some idea as to how much calcium is likely to be retained in the intestinal contents and how long this element is likely to go on increasing there during a period of bone-meal feeding may be obtained from the figures given for the calcium in the urine and feces of cows A–43 and A–46 in table 2. Cow A–37 is omitted from this discussion because she was badly off feed in the last of the periods in which bone meal was fed. It will be seen that the calcium content of the urine and feces of cows A–43 and A–46 did not reach its height until the second or third week of bone-meal feeding.

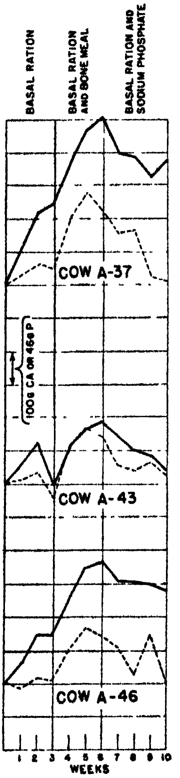

FIGURE 2.—Fluctuation in the content of calcium and phosphorus in the bodies of the cows during the course of the second experiment. The solid line represents calcium; the dotted line, phosphorus. The divisions on the ordinate correspond to 100 grams of calcium or 46 grams of phosphorus, the ratio in which these elements are present in bone.

Unfortunately the percentage of calcium in the feces alone was not determined. However, the percentage of calcium in the amount of feces and urine discharged weekly and mixed with a small amount of wash water was determined; and, as the urine contains very little calcium as compared with the feces, these figures give a rough idea of the amounts of calcium contained in the intestinal contents at the beginning and end of the bone-meal feeding period. The actual amount of calcium contained in the fill at the beginning of this period, and the actual increase during its progress would, of course, be considerably larger than these figures indicate.

The average amount of calcium in the urine and feces of cows A–43 and A–46 during the last period that they were on the basal ration was 0.238 percent, and during the last period on bone meal it was 0.378 percent. On the supposition that the fill amounted in each case to 100 kilograms, this would mean that the intestinal contents of the cows had 238 grams of calcium at the beginning of the bone-meal feeding period and 378 grams at the end, and that the calcium retained in the intestinal tract increased by 140 grams during this period. This increase accounts for most of the increase in body calcium as shown by the balance results. It is not unreasonable to suppose, therefore, that the positive calcium and phosphorus balances in the second period of the experiment represent merely an accumulation of these elements in the intestinal contents; and that the negative balances in the third period represent a gradual loss of this accumulation after the daily intake had been decreased.

It is not unlikely that a somewhat similar explanation may account for the changes in the balances which took place in the first, second, and third periods of the first experiment where the intake of calcium was reduced by the feeding of timothy hay. This interpretation would not appear to invalidate the conclusions expressed in respect to the different phosphate supplement periods where the intakes of calcium and phosphorus were maintained quite uniformly constant.

SUMMARY AND CONCLUSION

Two experiments made to determine the relative value of certain soluble phosphates and bone meal as phosphorus supplements in dairy rations, are reported. In the first experiment the phosphorus supplements were orthophosphoric acid, monosodium, disodium, and trisodium phosphates; in the second experiment bone meal was used. During the first period of each experiment the same basal ration was fed, but the first experiment was started in late September, when hot weather and annoyance from flies reduced the consumption of feed, whereas the second experiment was started in the cooler weather of November. The result was smaller intakes of calcium and phosphorus and negative balances in the first experiment and larger intakes of calcium and phosphorus and positive balances in the second experiment.

Dairy rations of alfalfa hay and grain often have a rather large proportion of calcium as compared with phosphorus. The experiments here reported indicated that calcium and phosphorus balances of cows fed on such rations may sometimes be rendered more positive by adding orthophosphates to them. This improvement is more marked when the nearly neutral phosphates disodium phosphate, and monosodium phosphate are used than when orthophosphoric acid or trisodium phosphate is used.

Large increases in the amount of calcium and phosphorus received by cows in their rations are likely to be followed by more positive calcium and phosphorus balances; decreases, by less positive balances.

There is reason to believe, however, that considerable quantities of calcium and phosphorus may be retained as insoluble tricalcium phosphate for several weeks in the intestinal tracts of cows, and there is no way of knowing what proportion of positive calcium and phosphorus balances is to be explained in this way and what proportion really represents a gain in bone tissue. Changes in the balances, following large changes in the calcium and phosphorus intake, and lasting not more than a few weeks, should not therefore be taken as any certain indication of changes in the assimilation of calcium and phosphorus from the intestinal tract.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT IN CERTAIN WHEAT CROSSES[1]

By D. C. TINGEY, *assistant agronomist*, and BION TOLMAN, *graduate student, Utah Agricultural Experiment Station*[2]

INTRODUCTION

During recent years the principles of Mendelism have been applied extensively in the production of new types of plants possessing resistance to various diseases. The results of this mode of attacking the disease problem have been highly favorable. Old varieties are gradually giving way to newer types equal to or exceeding the old in quality and productivity as well as possessing resistance to one or more diseases.

Loose smut in wheat, *Ustilago tritici* (Pers.) Jens., while not as serious a problem in Utah as the covered smut (*Tilletia* spp.) has, according to Tapke (*16*)[3] caused an average annual loss in this State of between 50,000 and 100,000 bushels of wheat (fig. 1). The various methods advocated for the control of loose smut in wheat, with the exception of the use of resistant varieties and hot-water treatments, have been either impractical of application or ineffective in control, or both (*16*).

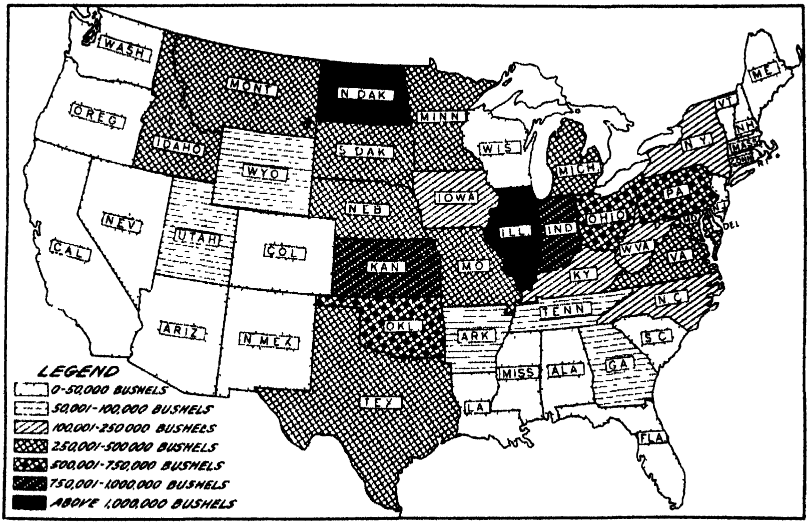

FIGURE 1.—Estimated average annual loss of wheat due to loose smut, by States, 1917–26, inclusive. From Journal of Agricultural Research 39, 314 (1929).

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[2] The results reported herein were obtained in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture. The writers are indebted to R. J. Evans, agronomist and A. L. Wilson, associate horticulturist, of the Utah Agricultural Experiment Station; to V. H. Tingey, assistant professor of mathematics, of the Utah State Agricultural College; and to F. A. Abegg, associate geneticist, Division of Sugar Plant Investigations, U.S. Department of Agriculture, who critically read the manuscript. Acknowledgment is made to V. F. Tapke for the use of figures 1 and 2. The writers wish also to express their appreciation to Margaret Richards Cook, who calculated the data in the tables.

[3] Reference is made by number (italic) to Literature Cited, p. 655.

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According to Tapke (*16*) the modified hot-water treatment devised by Freeman and Johnson (*4*) has generally been the method recommended. While this method is effective, if properly carried out, it is rather complicated and tedious to apply, especially for farmers, who usually are not properly equipped. Because of this and the fact that the disease frequently escapes observation, seed treatment for the control of loose smut is seldom practiced; as a result, the disease is allowed to go unchecked. The development of a resistant variety possessing the other desirable characteristics of locally grown spring wheats would be a decided advantage to the farmers in combating the disease.

REVIEW OF LITERATURE

A number of studies have been made on the comparative resistance of wheat varieties to loose smut. Tapke (*16*) gives an account of his studies on varietal resistance to this disease. It is evident from his work, as well as that of others, that some varieties exhibit greater resistance than others. Matsuura (*12*) reviews a report, Washington Agricultural Experiment Station Bulletin 155, presumably on the inheritance of resistance to *Ustilago tritici*. However, the original article refers only to smut and does not state whether it was *U. tritici* or *Tilletia tritici*. An article by T. Kilduff (*9*) came to the attention of the writers as this manuscript was ready for publication. The writer was unable to give a genetic analysis of inheritance to loose-smut resistance.

EXPERIMENTAL MATERIAL AND METHODS

PARENTAL MATERIAL USED

Varieties and strains of wheat used in these studies were Hope C.I. 8178; Preston C.I. 3081; 01–24, C.I. 11542; Dicklow No. 3; and Federation. These are all classed as *Triticum vulgare* Vill. wheats. The two leading spring varieties grown in Utah are Federation and Dicklow. Dicklow No. 3 is a Utah selection out of the Dicklow variety. This strain is the one used as a check in the wheat-nursery tests conducted at the station. It is more uniform, is less subject to lodging, and is a slightly higher yielder than the ordinary Dicklow variety. Strain 01–24 is a new production from the Utah station. It is a strong-strawed, high-yielding, white-kerneled spring wheat. The parents of this strain are not definitely known. However, it is probably a segregate out of either a Dicklow × Federation or a Dicklow × C.I. 4722 cross. The reaction of this strain to loose smut places some doubt on the possibility of its being out of the Dicklow × Federation cross, as Federation appears to possess no factors for resistance and 01–24 is more resistant than the Dicklow No. 3 strain. There is, however, the possibility that the Dicklow or Federation used in the cross from which 01–24 might have been selected was more resistant than the Dicklow No. 3 or the Federation used in these studies. Selections of Dicklow and Federation made at the Utah station show definitely that these two varieties do possess individuals differing in physiological characters; similar or even greater differences are possible in regard to their reaction to loose smut. A history of the origin and development of Hope C.I. 8178 is given by McFadden (*10*); Preston C.I. 3081 and Federation are described by Clark et al.

(*1*). The varieties, with contrasted characters studied, are shown in table 1.

TABLE 1.—*Contrasted characters of the parents studied in the crosses*

Parental variety	Morphological characters			Average percentage of infection with loose smut
	Presence or absence of awns	Chaff color	Grain color	
Hope	Fully awned	White	Red	0
Preston	do	do	do	1. 6±0. 39
01-24	Short apical awns	Bronze	White	18. 55±1. 21
Dicklow No. 3	do	White	do	38. 47±1. 27
Federation	Short beaks	Bronze	do	73. 50±1. 57

Inoculation experiments conducted in 1928 showed Hope wheat to be completely immune to the inoculum used. Later McFadden (*10*), who is responsible for the development of this variety, was led to conclude from his observations and tests that Hope wheat is highly resistant, if not immune, to the loose smut occurring on Kota. Other strains and varieties were found to possess varying degrees of resistance. However, Dicklow and Federation were found to be susceptible to the inoculum used. Federation was highly susceptible, whereas Dicklow possessed only a fair degree of resistance. This was somewhat surprising as Dicklow has consistently smutted under natural conditions more than Federation. However, it is undoubtedly due to a type of morphological resistance possessed by the Federation and not by the Dicklow variety. This resistance appears to be of such a nature as to prevent the smut spore from coming in contact with the stigma, such as having closer flowering glumes or having stigmas which are less likely to protrude outside the flowering glume during the blooming period.

INOCULUM USED

One of the difficulties in attempting to place the inheritance of disease resistance on a definite factorial basis is the possibility that the inoculum used may not be of a single physiologic form.

Rodenheiser (*14*) concluded from culture studies on nutrient media that there were physiologic forms of *Ustilago tritici* and *U. nuda* (Jens.) K. and S. In fact, he is of the opinion that *U. tritici* and *U. nuda* are physiologic forms rather than separate species. Whether these or other forms will be different pathogenically remains to be determined. However, there is reason to believe that there are different physiologic forms of *U. tritici*. Humphrey and Tapke (*8*) conclude from cross-inoculation experiments that wheat and rye smuts were identical *U. tritici*. Reed (*13*) reports physiologic strains within *U. avenae* (Pers.) Jens. Faris (*2*) has reported similar results on *U. hordei* (Pers.) K. and S. Tisdale and Johnson (*17*) and Stakman and Christensen (*15*) have demonstrated the existence of physiologic strains of *U. zeae* (Beckm.) Ung. The inoculum used was originally taken from the Dicklow variety, and it apparently spread to Federation and Sevier. Dicklow has been grown at the Utah station for years, and the other varieties, except Federation and Sevier, have shown little or no infection. Some preliminary data secured seem to indicate that the inoculum used in these studies was comparatively uniform pathogenically. In figure 2 are shown two spikes of wheat, the one healthy and the other infected with loose smut.

EXPERIMENTAL METHODS

Pure-line crosses between Hope × Federation, Hope × Dicklow No. 3, and Preston × 01–24 were made at the Central Experimental Farm (North Logan) in 1928. Pollen from anthers of a single spike was used to pollinate the stigmas of a single spike. The progeny of a single F_1 plant were seeded in the spring of 1929. The kernels were spaced about 2.5 inches apart in a row. Inoculations were made by the time the spikes reached the full bloom stage. In preparing the heads for inoculation, the center and the basal and terminal florets of each spikelet were removed; if awns were present, they were clipped off. The glumes of the remaining florets were then spread apart with small hand forceps, and the stigma was thoroughly dusted with the inoculum. Seedlings were made from each F_2 plant. The number of F_2 plants represented in F_3 rows are shown in the various goodness-of-fit tables. The F_3 rows were sown in randomized blocks with duplicate plantings of the Hope × Federation cross and three replicates of the Hope × Dicklow No. 3 and Preston × 01–24 crosses. A number of parental rows were sown at random over the experimental area. The genetic characters studied were resistant to loose smut, awns, glume, and kernel color. Smut-infection data were based on plant count and not on head count.

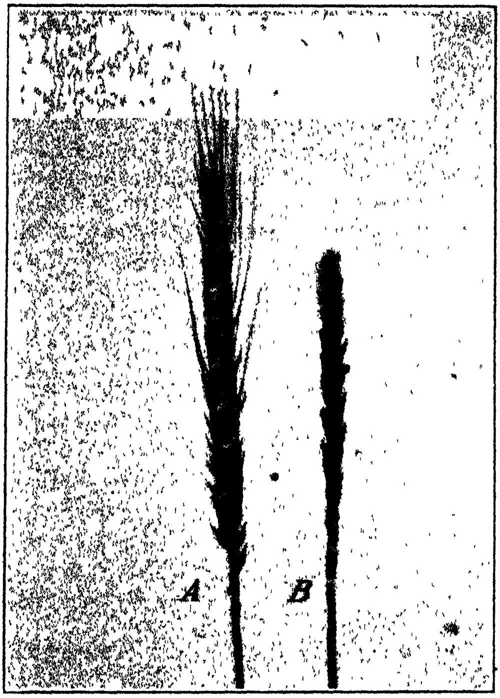

FIGURE 2.—*A*, Healthy spike of wheat; *B*, smutted spike. (From Journal of Agricultural Research, 39. 316 (1929).

In studying the goodness of fit, the χ^2 test, as given by Fisher (*3*), was used.

EXPERIMENTAL RESULTS

PRELIMINARY EXPERIMENTS ON INOCULATION TECHNIC

Some preliminary studies were made in 1928 in order to determine the proper time and method of inoculation to insure maximum infection.

METHODS OF INOCULATION

Chlamydospores were suspended in water and then placed on the stigmas of some of the plants; the powdered inoculum was placed on the stigmas of others by opening the glumes and applying the spores with a pair of forceps. The third method tried was that of heavily dusting the dry spores on the spikes of plants; and the fourth method was that of dipping the spike in a beaker of water heavily laden with spores of loose smut. Table 2 gives the results of this test.

TABLE 2.—*Percentage of infection obtained from various methods of inoculation (given to the nearest whole percent)* [a]

Method of inoculating	Federation		Dicklow No 3	
	Total plants	Plants infected	Total plants	Plants infected
	Number	*Percent*	*Number*	*Percent*
Spores placed on stigma				
Dusted on	73	60	54	52
Suspended in water	21	50	11	57
Spores placed on the spikes				
Dusted on	59	15	72	34
Suspended in water	22	5	12	0
Check (no inoculation)	17	0	15	0

[a] Data in this table show little difference in the percentage of infection in Dicklow No 3 and in Federation. The percentage of infection is somewhat high for one and correspondingly low for the other as compared with data in table 1 This is probably due to the fact that the data here are based on single 6-foot rows subject to rather wide variations, whereas data in table 1 represent an average for a number of rows.

It is evident from table 2 that the most effective methods of inoculating were those which involved the placing of the spores directly on the stigmas. However, there was no difference between placing the spores on the stigmas when dry or suspended in water. Dusting the spikes with the spores appeared more effective than dipping them in water containing spores. It will be seen from table 3 (compare with table 4), however, that response to dusting spikes with the inoculum varied with the variety and, therefore, could not be relied upon to give satisfactory results in genetic studies.

TABLE 3.—*Reaction of the wheat varieties and strains to loose smut when the inoculum was dusted on the spikes*

Wheat variety or strain	Total plants	Plants infected	Infection in check plants [a]	Wheat variety or strain	Total plants	Plants infected	Infection in check plants [a]
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
Dicklow	72	34.7	0.0	Q-250	105	1.9	2.4
Dicklow No 3	53	34.0	6.1	R-18-5	77	10.4	.0
Dicklow No. 16	61	41.0	.0	R-48-22	82	2.4	0
Federation	59	15.2	.0	R-S 4-5	106	7.4	.0
Hard Federation	60	0	0	R-S 17	72	4.1	0
Marquis	54	3.6	0	A-4	70	.0	0
Alcalde	58	.0	0	14-85	81	1.2	.0
Onas	52	13.0	0	G-40	60	.0	0
Sevier A	37	27.0	.0	G-43-11	75	4.0	.0
Sevier 59	36	16.7	.0	G-48	62	6.4	.0
Sevier 125	31	6.5	.0	G-149	65	3.1	.0
No. 139-3	59	11.9	.0	4-287	67	3.0	.0
No. 146	57	7.0	1.2	5-185	68	7.3	1.0
No. 49-10	43	4.7	.0	9-7	48	10.5	.0
No. 1-174-2	68	10.3	1.6	11-12	80	13.7	.0
01-24	47	10.6	.0	11-88	74	1.5	.0
Q-80	91	6.6	.18	12-101	65	.0	.0
Q-227	80	3.7	.0	13-47	83	3.5	.0
Q-231	68	1.4	.0				

[a] Checks not inoculated.

TIME OF INOCULATION

Inoculations were made at three different stages of anthesis: (1) When the stamens were green, (2) when they were yellow, and (3) when the pollen was being shed. In all cases the smut was placed directly on the stigma with forceps. Data on the time of inoculation are given in table 4. Student's pairing method (*3*) was used to determine whether there were any significant differences. It is obvious from the probability, as shown by *P* in these tables, that there are no significant differences; at least, if there are any, they are covered up by the differential reaction of the varieties.

TABLE 4.—*Percentage of loose-smut infection obtained on different varieties and strains inoculated at two different stages of anthesis and the probability of a difference in the two means*

GREEN AND YELLOW STAMENS

Wheat variety or strain	Percentage of infection when plants were inoculated while stamens were—		Wheat variety or strain	Percentage of infection when plants were inoculated while stamens were—	
	Green	Yellow		Green	Yellow
Alcalde	100 0	100.0	R-S 4-5	77 8	76 9
Onoas	95.0	66 7	R-S-17	77 8	100 0
Sevier 125	60.0	80 0	11-30	70 0	[a] 80.0
1-46	73.7	90.5	Dicklow No. 3	83.3	64 4
Q-248	50.0	60.0	Federation	80.0	[b] 79 80
II-R-18-5	80.0	88 9			
R-48-22	63.6	60 6	Mean	75 9	79 0

STAMENS GREEN AND STAMENS SHEDDING POLLEN

	Green	Shedding pollen		Green	Shedding pollen
Marquis	33.3	33.3	Dicklow No. 3	83.3	[c] 50 0
Q-227	81.8	30.8	Federation	80.0	[d] 89 6
9-7	87.5	78.6			
14 61	22 2	31.6	Mean	64.7	52 3

STAMENS YELLOW AND STAMENS SHEDDING POLLEN

	Yellow	Shedding pollen		Yellow	Shedding pollen
01-24	81.2	81.2	Dicklow No. 3	64.4	[e] 50 0
Q-89	90.9	65.0	Federation	79.8	[f] 89.6
F-68	36.4	52.9			
11-12	81.8	25.0	Mean	73 1	66.2
11-88	76.9	100.0			

[a] $t=0.71$. [b] $P=0.5$. [c] $t=1.23$. [d] $P=0.2$–0.3. [e] $t=0.65$. [f] $P=0.5$–0.6.

The data presented in table 4 at first seemed to indicate that more infection was obtained when the stamens were green, as was stated by Tapke (*16*). However, when the data were analyzed statistically, no significant difference was noted between the three periods of inoculation. Maddox (*11*) states that the time of maximum infection is during the period when the pollen is being shed. Freeman and Johnson (*4*) concluded that maximum infection occurs during the period of full bloom and that some degree of infection occurs until the ovary has reached one third its mature size.

STUDIES ON INHERITANCE OF RESISTANCE TO LOOSE SMUT

Difficulties in Placing Resistance on a Definite Factorial Basis

Several difficulties are encountered in attempting to place on a definite factorial basis the inheritance of resistance to loose smut, as well as to any other disease. One of the most complicating factors is the effect of environment. This effect was partly reduced by replication. Some comparatively susceptible types occasionally escape the disease when grown in short rows, even though artificially inoculated. For example, Preston on the average smuts about 1.6 percent, with some rows smutting as high as 9.5 percent; however, 70 percent of the rows, in these studies with 30 seeds sown in each row, entirely escaped infection.

The possibility of the inoculum not being entirely uniform, because of the possible existence of physiologic forms of loose smut, also adds to the difficulty of placing the inheritance of resistance on a definite factorial basis. In spite of these complications, an attempt has been made to place resistance on a Mendelian basis.

Relation of Infection to Sheath Color

An interesting condition developed on the sheaths and the exposed culms of inoculated plants. Usually the plants with smutty spikes developed a distinct grayish-purple color on the leaf sheaths. At first it appeared as if the coloration were a characteristic of the culm; on closer examination it was found that the coloration was generally confined to the sheath portion of the leaf. It was also found on the portion of the culms exposed to the light. This peculiar coloration developed on Hope and Preston during the year they were inoculated, whereas on Federation no color developed even though the plants smutted. In the F_3 generation there appeared to be a segregation for this condition, suggesting that it may partly be controlled by genetic factors. According to Heald (*7, pp. 683 684*), a similar condition was observed by McAlpine, who stated that "when a stool is affected with loose smut, the stalks are generally of a purplish tint, so that they can be readily picked out from among the general crop."

Biometrical Studies

RELATION OF SMUT INFECTION AND SEEDLING MORTALITY

In the study of disease resistance where the disease organism is operative during the seedling stage of the host plant, it is important to know whether or not there is any differential relationship between the infection and seedling mortality among resistant and susceptible lines. If such a relationship existed, it would no doubt materially complicate a genetic interpretation of inheritance. In order to determine whether this condition did exist, a known number of kernels were seeded in each F_3 row. This made it possible to calculate the percentage of seedling plants reaching maturity, or the percentage stand. It appeared evident that if the disease were causing the death of any appreciable number of susceptible seedlings, there should be a relationship in the percentage of smut obtained in the F_3 rows and the percentage stand. Simple correlation coefficients were used to measure whether or not a relationship existed; since F_3 rows were replicated, an average of the replication was taken for both the percentage of smut and the percentage of stand; this average replication was used in calculating the correlations. Correlation coefficients thus obtained, between the percentage of smut and the percentage of stand for the susceptible parental strain and for the F_3-progeny rows, are shown in the following tabulation:

Material:	*r*
Federation	0.09±0.167
Dicklow No. 3	.10± .115
01–24	.16± .096
Hope × Federation (F_3 rows)	.02± .047
Hope × Dicklow No. 3 (F_3 rows)	.02± .039
Preston × 01–24 (F_3 rows)	.01± .039

There seems to be no evidence from these data that smut has any differential influence on the percentage stand in the F_3 rows of resistant and susceptible lines. This is further shown in considering the average percentage stand obtained in the F_3 parental rows, since here it is possible to compare the inoculated resistant and susceptible strains. The average percentage stand was: Hope, 65.1; Preston, 54.6; 01–24, 56.2; Dicklow No. 3, 68.5; and Federation, 75.2.

Thus it appears safe to conclude that under the conditions of the experiment, the smut organism had no greater effect on the seedling mortality, on an average, in susceptible than in resistant lines.

DETERMINING THE CLASS INTERVAL

Because of the unknown experimental errors in percentage of infection occurring from single-row plantings, it seemed advisable to make replicate seedings and to determine the size of the errors in the various crosses, and use these results in interpreting the data. The experiments were planned with a view to using Fisher's (*3*) analysis-of-variance method, and the replicates were randomized accordingly. In two of the crosses there was enough seed for three replications, while in the other cross duplicate seedings only were possible. Analysis-of-variance data for the three crosses are given in table 5.

Fisher's (*3*) *Z* test was made to determine whether or not there was any treatment effect; the value of *Z* thus calculated is shown at the bottom of the last column of table 5. Since Fisher's (*3*) tables do not give the value for n_1 and n_2 as occurring in the analysis-of-variance tables, it was necessary to calculate this value from the formula given by him. This value is shown at the bottom of the table. The *Z* quantity, calculated by comparing the variance due to treatment with that due to error, as shown in table 5, is larger in all cases than *Z* for the 1-percent point. This shows that there is undoubtedly a treatment effect, which naturally was to be expected from the nature of the material, since the mean percentage of smut occurring in the replicated F_3 rows ranged from no smut to a rather high percentage.

TABLE 5.—*Analysis of variance for three wheat crosses*

HOPE × FEDERATION CROSS

Variance due to	Degrees of freedom	Sum of squares	Mean square	Half log
Replication	1	0.03		
Treatment	206	95,176.77	462.02	3.0678
Error	206	11,459.99	55.63	2.0090
Total	413	106,636.79		Z=1.0584

n_1=206 n_2=206 Z 1 percent point=0.1625

PRESTON × 01 24 CROSS

Variance due to	Degrees of freedom	Sum of squares	Mean square	Half log
Replication	2	571.09		
Treatment	306	39,743.41	129.88	2.4335
Error	614	44,335.41	72.44	2.1414
Total	922	116,636.79		Z=.2937

n_1=306 n_2=614 Z=1 percent point=0.1148

HOPE × DICKLOW

Variance due to	Degrees of freedom	Sum of squares	Mean square	Half log
Replication	2	149.91		
Treatment	306	78,482.39	256.48	2.7736
Error	614	37,498.94	61.27	2.0579
Total	922	116,131.24		Z=.7157

n_1=306 n_2=614 Z 1 percent point=0.1146

It is evident from table 5 that the variance of a single determination is 55.63 for the Hope × Federation cross, 72.44 for the Preston × 01 24 cross, and 61.27 for the Hope × Dicklow No. 3 cross. These values were used in each case to set up a difference necessary between two mean percentages of infection for a probability of 0.05. Since the error was based on a rather large number, a significant difference would amount to about twice the standard error of the difference. This quantity amounted to about 13 percent in the Hope × Dicklow No. 3 cross, 14 percent in the Preston × 01 24 cross, and 15 percent in the Hope × Federation cross. Then, in classifying the F_3 rows, the value necessary for a significant difference was taken as the class interval; on this basis a frequency table was constructed. The proportion of F_3 rows falling into each of the classes and the reaction of the parental types formed the basis for arriving at the factorial explanation for the inheritance of resistance to loose smut herein suggested.

GENETIC STUDIES OF RESISTANCE TO LOOSE SMUT

The reaction of the parental material to loose smut (*Ustilago tritici*), as shown in table 6, seemed to indicate that possibly more than one factor was involved in the inheritance of resistance. This was also suggested by the breeding behavior of the F_3 progeny of the various crosses. On the basis of these two conditions, it was assumed that at least three factors were involved in the inheritance of resistance to loose smut. This factorial relationship of the parental material is shown in table 6.

Since Hope has never smutted even though thousands of inoculated plants have been grown, it was considered to be completely immune to the inoculum used; therefore, it possessed all three factors in the dominant condition, though dominance is evidently incomplete and the factors have a cumulative effect. Likewise, each of the three factors are thought to have a different effect, an individual with the R_2R_2 factor showing somewhat more resistance than one with the R_3R_3 factor and one with the R_1R_1 factor being about as resistant as one possessing the other two factors. This does not mean that these factors have definite numerical values with specific expression, regardless of the genotype, as factor interaction is not an uncommon phenomenon.

On an average, Preston has smutted about 1.6 percent, although when it is grown in rows of approximately 30 seeds to the row, similar to the F_3-progeny rows, about 70 percent of the rows show no smut at all; other rows show as much as 9.5 percent smut. Consequently, it was assumed that Preston lacked one of the factors common to Hope and that the absence of this factor allows some smut to develop. Preston would then be classed as highly resistant but not immune, as was Hope. Under similar conditions, 01–24 smuts about 20 percent. Therefore, it was assumed that it possesses two factors in common with Hope but that it differs from Preston in two factors.

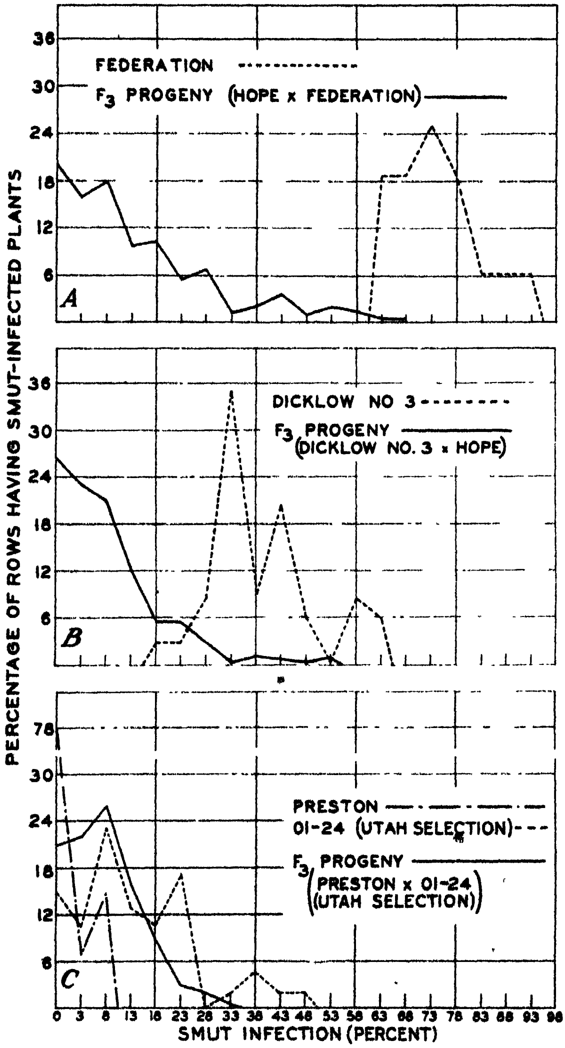

FIGURE 3.—Frequency distribution of parental varieties and F_3 progeny in various wheat crosses as related to loose-smut infection. *A*, Federation and F_3 progeny of Hope × Federation; *B*, Dicklow No. 3 and F_3 progeny of Dicklow No. 3 × Hope; *C*, Preston, 01-24 (Utah selection) and F_3 progeny of Preston × 01-24 (Utah selection).

Dicklow No. 3 apparently was more susceptible than 01–24 and, therefore, was assigned only the one factor, R_3R_3; lacking R_1R_1 and

R_2R_2, it is comparatively susceptible and smuts on an average nearly 40 percent.

TABLE 6.—*Genetic composition assigned each of the parental types, on the basis of reaction to loose smut, the range, and the average percentage of infection occurring in rows of 30 kernels each*

Parent	Genotype	Rows	Infection	
			Range	Average
		Number	*Percent*	*Percent*
Hope C.I. 8178	$R_1R_1\ R_2R_2\ R_3R_3$	50	0–0	0
Preston C.I. 3081	$R_1R_1\ R_2R_2\ r_3r_3$	26	0–9.5	1.6±0.39
01-24 C.I. 11542	$r_1r_1\ R_2R_2\ R_3R_3$	47	0–50.0	18.5±1.21
Dicklow No. 3	$r_1r_1\ r_2r_2\ R_3R_3$	34	18.2–61.9	38.5±1.27
Federation	$r_1r_1\ r_2r_2\ r_3r_3$	16	60.9–91.3	73.5±1.57

Federation is assumed to possess none of the factors for resistance, since it ordinarily smuts 70 percent or more.

In table 7 is shown the distribution of the parents and the F_3 of the various crosses in 5-percent classes for loose-smut infection, and figure 3 is a graphical presentation of the same data.

HOPE × FEDERATION CROSS

It is evident from the factorial relationship assigned each parental type that the Hope × Federation cross should give rise to 27 F_2 genotypes. The reaction of the known genotypes, the parents, to the smut inoculum is shown in table 6. On the basis of these known types, the behavior of the remaining genotypes was formulated.

TABLE 7.—*Distribution of parents and F_3 rows of the crosses named in 5-percent classes for loose-smut infection*

Parent or cross	Rows smut-free	Rows having percentages of loose-smut infection falling within the indicated 5-percent classes (average of three replications for F_3, single rows for parents)																			Total rows
		3[a]	8	13	18	23	28	33	38	43	48	53	58	63	68	73	78	83	88	93	
Hope C.I. 8178 number	16																				16
Federation number	0													3	3	4	3	1	1	1	16
91b-Hope (C.I. 8178) × Federation number	43	33	38	20	22	12	13	3	4	7	3	4	3	1	1						207
Hope C.I. 8178 number	34																				34
Dicklow No. 3 (Utah selection) number	0				1	1	3	12	3	7	2	0	3	2							34
87b-Hope C.I. 8178 × Dicklow No. 3 (Utah selection) number	82	71	65	36	17	17	8	3	4	1	0	2									307
Preston C.I. 3081 number	20	2	4																		26
01-24 (Utah selection) number	7	5	11	6	5	8	0	1	2	1	1										47
93b-Preston × 01-24 (Utah selection) number	65	71	81	46	27	9	7	1													306

[a] Taken to the nearest whole number.

The basic phenotypic ratio ordinarily obtained in F_2 in a cross, when three independent factors expressing different characters are involved, is 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1. This ratio, however, may be modified by factor interaction. In the case under consideration the factors were assumed to be of unequal value and were all involved in the expression of a single character. It has been shown that strains even though somewhat susceptible, escape infection when sown in short rows and, therefore, would be placed in the nonsmutting group.

This would tend to make this group too large. To obviate this difficulty, those progenies showing no infection were included in the lowest frequency group. This class interval permitted a certain percentage of infection, which in this cross included those with no infection up to 14.9 percent. The method of arriving at the class interval has previously been discussed. The reason for using the experimental error in arriving at the class interval is that F_3 strains may actually be susceptible up to as much as 15 percent, and yet a certain proportion may escape infection when grown in short rows even though replicated. This would also be true if the interval were extended, but not with the same probability. The phenotypes, the theoretical parental types, and the basis of classification of the F_3 rows in the Hope × Federation cross are shown in table 8.

TABLE 8.—*Possible F_2 genotypes; the theoretical parental type; the percentage of infection, and the basis of classification of the F_3; also the phenotypic ratio in the Hope × Federation cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_3 classification		
				Class interval	Average infection[a]	Phenotypic ratio
			Percent	*Percent*	*Percent*	
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	0	0-14.9	6.9	45
$R_1R_1\ R_2R_2\ R_3r_3$	2					
$R_1R_1\ R_2r_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1R_1\ R_2r_2\ R_3r_3$	4					
$R_1r_1\ R_2R_2\ R_3r_3$	4					
$R_1r_1\ R_2r_2\ R_3R_3$	4					
$R_1r_1\ R_2r_2\ R_3r_3$	8					
Total	27					
$R_1R_1\ R_2R_2\ r_3r_3$	1	Preston	1.6			
$R_1R_1\ R_2r_2\ r_3r_3$	2					
$R_1r_1\ R_2R_2\ r_3r_3$	2					
$R_1r_1\ R_2r_2\ r_3r_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ R_3R_3$	1	Intermediate (Preston and 01-24)[b]				
$R_1R_1\ r_2r_2\ R_3r_3$	2					
$R_1r_1\ r_2r_2\ R_3R_3$	2					
$R_1r_1\ r_2r_2\ R_3r_3$	4					
Total	9					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01-24	18.5	15-29.9	19.9	12
$r_1r_1\ R_2R_2\ R_3r_3$	2					
$r_1r_1\ R_2r_2\ R_3R_3$	2					
$r_1r_1\ R_2r_2\ R_3r_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ r_3r_3$	1	Equal to 01-24[c]	18.5			
$R_1r_1\ r_2r_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ r_3r_3$	1	Intermediate (01-24 and Dicklow No. 3).[d]		30-44.9	34.9	3
$r_1r_1\ R_2r_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ R_3R_3$	1	Dicklow No. 3	38.5	45-59.9	46.5	3
$r_1r_1\ r_2r_2\ R_3r_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ r_3r_3$	1	Federation	73.5	60-74.9	62.3	1

[a] The average of the class and not the mid point.

[b] Not as resistant as Preston but more resistant than 01-24; somewhere between 1.6 and 18.5 percent.

[c] The R_1R_1 factor was assumed to be equivalent in effect to the R_2R_2 and R_3R_3 factors.

[d] Not as resistant as 01-24 but more resistant than Dicklow No. 3, somewhere between 18.5 and 38.5 percent.

On this basis, Hope, Preston, and the Intermediate (Preston and 01–24) genotypes all fell in the same class. It is evident from table 8 that forty-five sixty-fourths of the F_3 progeny rows would theoretically fall in the first phenotypic group, in which infection would range from 0 to 14.9 percent. In the second group infection ranged from 15 to 29.9 percent; this group included the genotypes corresponding to the 01–24 parental type. Inasmuch as the R_1R_1 factor carried by Hope is assumed to be equal in effect to the other two factors, twelve sixty-fourths of the progeny rows would be expected in the second-class interval. In the third group infection ranged from 30 to 44.9 percent. This group was composed of the genotypes which were neither 01–24 nor Dicklow No. 3 types but which fell somewhere between them; three sixty-fourths of the progeny rows should be of this type. The genotypes corresponding to the Dicklow No. 3 parental type constituted the fourth group, in which infection ranged from 45 to 59.9 percent; three sixty-fourths of the progeny rows would also be expected to be in this class. Infection in the last or upper group ranged from 60 to 74.9 percent; this group included the genotype characteristic of Federation and included only one sixty-fourth of the progeny rows. This completes the phenotypic ratio of 45 : 12 : 3 : 3 : 1. Table 9 shows the goodness of fit obtained when the observed data were fitted to this ratio. The probability is between 0.2 and 0.3 and is considered satisfactory.

Table 9.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Hope × Federation cross, based on a 45 : 12 : 3 : 3 : 1 ratio*

Smut (percent)	Number of progeny	
	Observed	Calculated
0 to 14.9	132	143.0
15 to 29.9	43	39.4
30 to 44.9	14	9.6
45 to 59.9	13	9.6
60 to 74.9	5	3.2

$\chi^2=5.4085$
$P=0.2-0.3$.

HOPE × DICKLOW NO. 3

It will be observed that Hope and Dicklow No. 3 differ from each other in two factors and that there will be no genotypes which do not carry the R_3R_3 factor for resistance. The possible F_2 genotypes, the theoretical parental types, the basis of classification of the F_3 rows, and the phenotypic ratio of Hope × Dicklow No. 3 cross are shown in table 10.

TABLE 10.—*Possible F_2 genotypes, the theoretical parental type, percentage infection, and the basis of classification of F_3, also the phenotypic ratio in the Hope × Dicklow No. 3 cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_3 classification: Class interval	F_3 classification: Average infection[a]	F_3 classification: Phenotypic ratio
			Percent	*Percent*	*Percent*	
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	0	0-12.9	6.8	12
$R_1R_1\ R_2r_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1r_1\ R_2r_2\ R_3R_3$	4					
Total	9					
$R_1R_1\ r_2r_2\ R_3R_3$	1	Intermediate (Preston and 01-24)[b]				
$R_1r_1\ r_2r_2\ R_3R_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01-24	18.5	13-25.9	17.1	3
$r_1r_1\ R_2r_2\ R_3R_3$	2					
Total	3					
$r_1r_1\ r_2r_2\ R_3R_3$	1	Dicklow No. 3	38.5	26-55.0	37.1	1

[a] The average of the class and not the mid point.

[b] Not as resistant as Preston but more resistant than 01-24; somewhere between 1.6 and 18.5 percent.

The amount necessary to give a significant difference between two mean percentage infections, calculated as previously stated (with a probability of 0.05) was approximately 13 percent. Consequently, this was the amount used as the class interval in separating the phenotypic groups and in determining the phenotypic ratio. As in the Hope × Federation cross, no attempt was made to differentiate between the genotypes resembling the Hope parent and those which carried the R_1R_1 and R_3R_3 factors, making them intermediate (Preston and 01-24) types, because they were all included in the same class interval. The range of smut infection allowed in the first phenotypic group was from 0 to 12.9 percent. The class interval in this cross is slightly less than that allowed for the corresponding genotypes of the Hope × Federation cross. This reduction in class interval was due to the slight difference in the variance obtained in this cross and also to the fact that there were triplicate plantings which would reduce the standard error of a difference accordingly. Inasmuch as the class interval is reduced 2 percent in this cross, the range of smut allowed by the various genotypes is correspondingly reduced. Infection in the second phenotypic group ranged from 13 to 25.9 percent and included the genotypes typified by the 01-24 parent. The upper class included all strains with 26 percent infection or more. There were only 20 of the 307 progeny rows that smutted above that amount. This was almost the exact number expected to conform to the Dicklow No. 3 genotype. Infection in the progeny included in this group ranged from 27.1 to 55 percent and averaged 37.05. This is about what would be expected of plants having a genetic make-up equivalent to Dicklow No. 3. For this reason all strains with 26-percent infection or over were considered as one class. This classification gave a phenotypic ratio of 12 : 3 : 1. Table 11 shows the goodness of fit obtained when the observed data were compared with the above ratio. It is evident from table 11 that the goodness of fit was again satisfactory.

TABLE 11.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Hope × Dicklow No. 3 cross, based on a 12:3:1 ratio.*

Smut (percent)	Number of progeny	
	Observed	Calculated
to 12.9	236	230.2
13 to 25.9	50	57.6
Above 26	20	19.2

$\chi^2 = 1.2169$
$P = 0.5$-0.7

In the Preston × 01-24 cross all the factors common to Hope are involved. However, the R_2R_2 factor is present in both parents; as a result, this factor appears in a homozygous dominant condition in all the genotypes obtained from the cross. Data relative to this cross are shown in table 12.

TABLE 12.—*Possible F_2 genotypes, the theoretical parental type, the percentage of infection, and the basis of classification of the F_3, as well as the phenotypic ratio in the Preston × 01-24 cross*

Genotype	Number of each	Theoretical parental type	Actual parental average infection	F_3 classification		
				Class interval	Average infection[a]	Phenotypic ratio
$R_1R_1\ R_2R_2\ R_3R_3$	1	Hope	*Percent* 0	*Percent* 0-13.9	*Percent* 5.8	12
$R_1R_1\ R_2R_2\ R_3r_3$	2					
$R_1r_1\ R_2R_2\ R_3R_3$	2					
$R_1r_1\ R_2R_2\ R_3r_3$	4					
Total	9					
$R_1R_1\ R_2R_2\ r_3r_3$	1	Preston	1.6			
$R_1r_1\ R_2R_2\ r_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ R_3R_3$	1	01-24	18.5	14-27.9	16.9	3
$r_1r_1\ R_2R_2\ R_3r_3$	2					
Total	3					
$r_1r_1\ R_2R_2\ r_3r_3$	1	Intermediate[b] (01-24 and Dicklow No. 3.)		28-41.9	32.0	1

[a] Average of the class and not the mid point

[b] Not as resistant as 01-24 but more resistant than Dicklow No. 3, somewhere between 18.5 and 38.5 percent.

PRESTON × 01-24

In conformity with the two previously discussed crosses, no distinction was drawn between the genotypes resembling the Hope parent and those resembling the Preston parent, because the range of smut allowed by each came within the range of the class interval. Thus, twelve sixty-fourths of the progeny rows were included in the first phenotypic group. The second group, ranging in infection from 14 to 27.9 percent, was made up of the genotypes resembling the 01-24 parent. Only 10 of the 307 F_3 rows smutted above 28 percent. These ranged in infection from 28 to 39 percent, with an average of 32, which is about as expected since the genotype of this phenotypic class carries the R_2R_2 factor, which should make it an intermediate (01–24 and Dicklow No. 3) type. From this classification a 12 : 3 : 1 ratio was expected; the goodness of fit obtained when the observed was compared with this ratio is shown in table 13.

TABLE 13.—*Goodness of fit obtained from the breeding behavior in F_3 in regard to loose-smut resistance in the Preston × 01–24 cross based on a 12 : 3 : 1 ratio*

Smut (percent)	Number of progeny	
	Observed	Calculated
0 to 13.9	237	230.2
14 to 27.9	60	57.6
Above 28	10	19.2

$\chi^2 = 4.7092$.
$P = 0.05–0.1$.

It is evident from table 13 that while the fit is not exceptionally good, nevertheless it is within the lower limits of probability usually set (0.05).

FACTORIAL RELATIONSHIP OF THE VARIOUS CROSSES

The fact that the same factors for resistance were assumed to be involved in all three crosses suggests that the progeny of similar genetic constitution obtained from the various crosses ought to give a similar reaction to the inoculum. This relationship is also shown in tables 8, 10, and 12. Infection in the phenotypic class of 45 in the Hope × Federation cross (table 8) ranged from 0 to 14.9 percent, with an average of 6.9; the corresponding class of 12 in the Hope × Dicklow No. 3 cross (table 10) ranged in infection from 0 to 12.9 and averaged 6.7; and the class of 12 in the Preston × 01–24 cross (table 12) ranged in infection from 0 to 13.9, with an average of 5.8. There is a rather close agreement in the mean percentages of infection in the three crosses for the lower class. The Preston × 01–24 cross should show a lower mean infection in the lower class because there were no intermediate (Preston and 01–24) types present. These types appearing in the other two crosses should be more susceptible than the Preston type. It will be noted (table 8, 10, and 12) that the average percentage of infection for the lower phenotypic group in all three crosses is slightly higher than for the Preston parent, which is most typical of the genotypes included in this class. This is to be expected in the Hope × Federation and the Hope × Dicklow No. 3, crosses, however, because the class interval is extended beyond that for Preston, and, therefore, includes intermediate (Preston and 01–24) types, which would be more susceptible than Preston; this would account for the higher average. The average of the lower class in the Preston × 01–24 cross is higher than expected. However, this does not appear especially serious as the same exactness in a study of disease resistance cannot be expected as in a study of morphological characters. This discrepancy may be due to any one or to a combination of the four following conditions:

(1) The heterozygous condition of some of the genotypes, which may allow for more infection than the homozygous, since dominance is not complete.

(2) The effect of modifying factors.

(3) The differential infection in parent and in F_3 progeny due to slight differences in the stage of inoculation. Although instructions were given to inoculate both parent and progeny before or soon after the anthers had shed their pollen (inoculations at later stages appears to reduce materially the amount of infection), it might have been possible that a number of Preston spikes were inoculated somewhat later, when the kernels were partly formed. This would lower the average percentage of infection of Preston. In this connection it might be well to mention that in 1933 Preston showed 9.5 percent of infection when inoculated with the same smut. It is not known whether this was due to differences in stages of inoculation or to soil and climatic differences.

(4) It is possible that one of the parents, especially Preston, was a mixed population. Therefore, the pure line of Preston used in this cross may have been slightly more susceptible than the average for the variety. This would necessitate a slight change in the genetic constitution of Preston from $R_1R_1R_2R_2r_3r_3$ to one of a type $R_1R_1r_2r_2$ R_3R_3 since the R_3R_3 factor allows more infection than the R_2R_2 factor. One would now expect the theoretical average of the first class to be considerably above 1.6 percent.

In view of these various possibilities, it appears desirable to leave the genetic composition of the varieties as shown in table 6.

The average percentage of infection for genotypes similar to 01–24 and Dicklow No. 3, in all crosses was within a few percent of these parental types (tables 8, 10, and 12). In the Hope × Federation cross (table 8) the 45 progeny observed in the upper class had an average of infection lower than that of the Federation parent, although they were within the range of the Federation; with the few progeny rows the differences may be due purely to error in sampling. In the Hope × Dicklow No. 3 cross no types were recovered with any higher range of infection than that of the Dicklow No. 3 parent. The 20 rows falling in this class had an average infection of 37.1 as compared with 38.5 for Dicklow No. 3. The Preston × 01–24 cross gave no genotypes which did not carry the R_2R_2 factor; hence, the highest infection expected was somewhere between the range of the parental varieties 01–24 and Dicklow No. 3. This would be somewhere between 18.6 and 38.5 percent. The average actually obtained for this class was 32 percent (table 12). In general, it appears as though the genotypes recovered from the various crosses corresponding to the parental types react to loose smut similarly to the parental types used and as though the similar genotypes recurring in the different crosses behave in a similar manner.

STUDIES ON THE INHERITANCE OF MORPHOLOGICAL CHARACTERS AND THEIR RELATION TO RESISTANCE TO LOOSE SMUT

Inheritance of Morphological Characters

The primary purpose in obtaining the breeding behavior of the awns, chaff, and kernel color was to determine whether or not there was any relationship between any of these morphological characters and disease resistance. The knowledge of such a relationship, if it did exist, would be of great value to the plant breeder.

AWNS

Hope × Dicklow No. 3 and Preston × 01–24 Crosses.—The awn inheritance of Hope × Dicklow No. 3 and of Preston × 01–24 crosses was similar. In both crosses one of the parents was fully awned, while the other parent had short, apical awns. The awns of the F_1 plants were of intermediate length, although they resembled

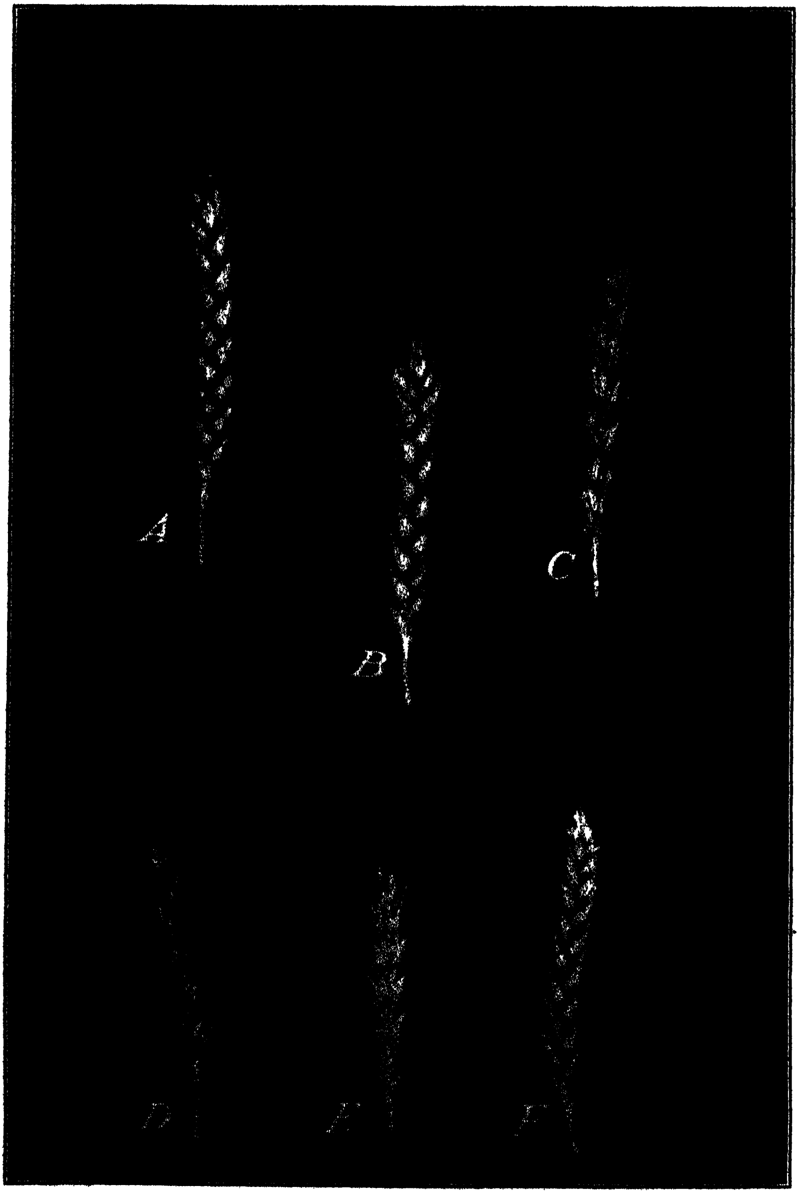

Figure 4.—Inheritance of awns in the Hope × Dicklow No. 3 and Preston 01–24 crosses: *A*, Short, apica awns typical of Dicklow No. 3 and 01–24; *B*, short, apical awns of the F_1; *C*, fully awned spikes common to Hope and to Preston; *D*, *E*, *F*, awn classes found in F_2

more closely the short-awned parent. Both parental types and a group of intermediates were recovered in the F_2 of each cross. The intermediates segregated in F_3; thus three distinct awn classes were obtained: (1) Short, apical awns, such as those in the Dicklow No. 3, and the 01–24 parents; (2) segregating types; and (3) fully awned specimens like the Hope and Preston parents. The parental, F_1 and F_2 types are shown in figure 4. Table 14 shows the observed

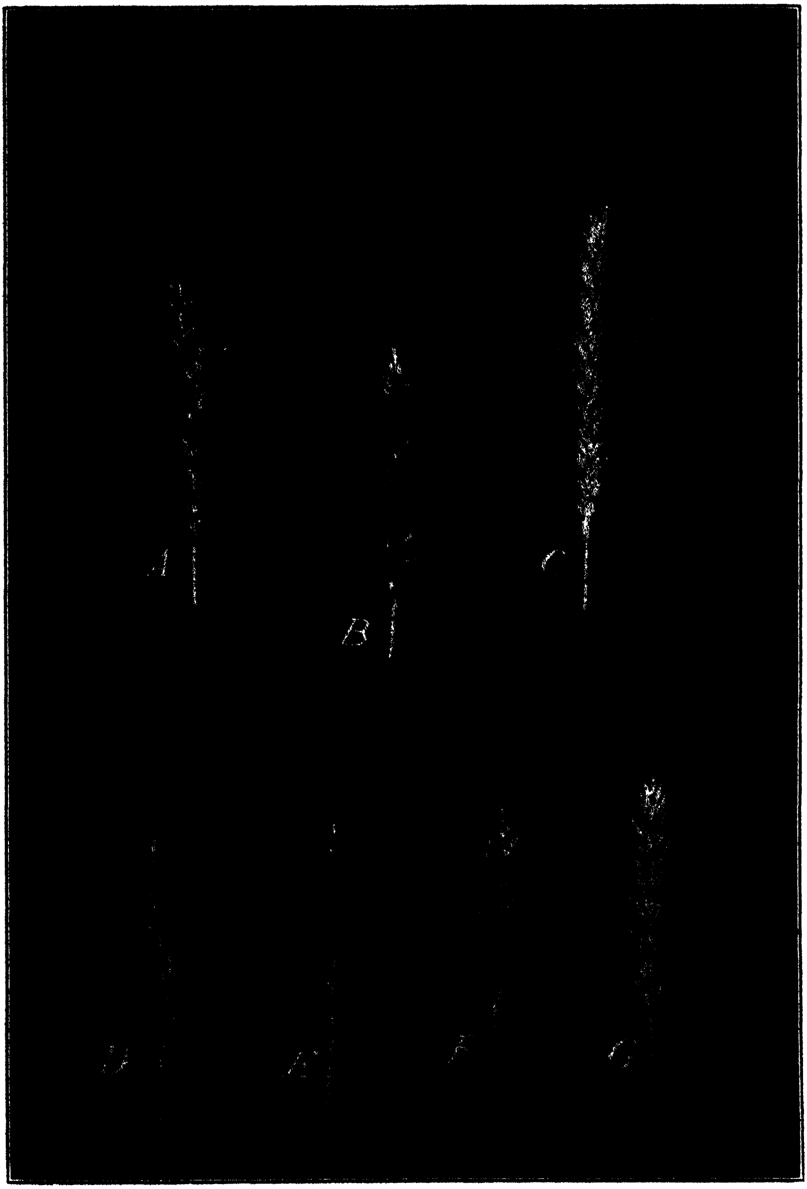

FIGURE 5.—Inheritance of awns in the Hope × Federation cross: *A*, Federation; *B*, F_1; *C*, Hope; *D*, *E*, *F*, *G*, awn classes found in F_2.

and calculated numbers falling in each class and the goodness of fit based on a 1:2:1 ratio. It is apparent from table 14 that a good fit was obtained in both crosses.

TABLE 14.—*F_3 breeding behavior of awns in the Hope × Dicklow No. 3 and the Preston × 01–24 crosses, and the goodness of fit based on a 1 : 2 : 1 ratio*

Parent [a]	Class	Observed	Calculated	χ^2	P
Hope (*AABB*) × Dicklow No. 3 (*aaBB*)	Short apical awns	82	76.7		0.7–0.8
	Segregating	150	153.3	0.4890	
	Fully awned	75	76.7		
Preston (*AABB*) × 01-24 (*aaBB*)	Short apical awns	78	76.7		
	Segregating	158	153.4	.5835	.7–.8
	Fully awned	71	76.7		

[a] Awnedness was arbitrarily chosen to be represented by the dominant characters, awnlessness might just as appropriately be so designated.

HOPE × FEDERATION CROSS.—The inheritance of awns in the Hope × Federation cross was quite different from that in the Hope × Dicklow No. 3 and Preston × 01–24 crosses just discussed. The F_1 in the Hope × Federation cross was intermediate in inheritance but again resembled most closely the awnless parent. The parental types, the F_1, and the true-breeding F_3 types of the Hope × Federation cross are shown in figure 5. Besides the four homozygous types shown, there were five segregating classes of progeny: (1) Those segregating for awn classes 1 and 2; (2) those segregating for awn classes 1,2, and 3; (3) those segregating for awn classes 1, 2, 3, and 4; (4) those segregating for awn classes 2, 3, and 4; and (5) those segregating for awn classes 3 and 4. There were, therefore, nine genotypic classes into which the F_3 progenies were classified. This breeding behavior suggested a two-factor difference with independent segregation. The relation of the observed to the calculated based on a two-factor difference and the closeness of fit to a 1:2:2:4:1:2:1:2:1 ratio is shown in table 15. It is evident from this table that a good fit was obtained.

TABLE 15.—*Breeding behavior of awns in the Hope × Federation cross and the goodness of fit to a 1 : 2 . 2 : 4 : 1 : 2 : 1 : 2 : 1 ratio*

F_3 breeding behavior	Observed	Calculated	χ^2	*P*
True breeding 4	15	13.0	3.3077	0.90–0.95
Segregating 3, 4	20	26.0		
Segregating 2, 3, 4	25	26.0		
Segregating 1, 2, 3, 4	50	52.0		
True breeding 3	16	13.0		
Segregating 1, 2, 3	25	26.0		
True breeding 2	14	13.0		
Segregating 1, 2	26	26.0		
True breeding 1	16	13.0		

KERNEL COLOR

Kernel-color inheritance was involved in all three crosses. The F_1 plants all had red grain and segregation took place in F_2.

HOPE × FEDERATION AND HOPE × DICKLOW NO. 3 CROSSES.—The proportion of white to red kernels in the F_2 in the Hope × Federation and Hope × Dicklow No. 3 suggested a three-factor difference, with each factor either alone or in combination expressing the character.

In the Hope × Dicklow No. 3 cross 5 plants out of 307 had white grain; it was assumed to be similar to the Hope × Federation cross, which had 4 white-kerneled plants out of 206 F_2's. Therefore, the Hope × Dicklow No. 3 cross was not studied for grain color in the F_3.

Studies made on the inheritance of kernel color in F_3 in the Hope × Federation cross behaved as would be expected from a study of the F_2 data. With a three-factor difference, the F_3 should theoretically segregate into 5 classes, giving a 37 : 12 : 8 : 6 : 1 ratio. The five classes into which the F_3's were classified, based on kernel color, are shown in table 16. In this table is also shown the observed number in each class which were fitted to a 37 : 12 : 8 : 6 : 1 ratio with a good fit resulting, as shown by the χ^2 test.

TABLE 16.—*Breeding behavior for grain color in the Hope × Federation cross and the goodness of fit based on a 37 : 12 : 8 : 6 : 1 ratio*

Class	Number of progeny		χ^2	P
	Observed	Calculated		
True-breeding red	122	118.4	0.7941	0.9–0.95
Segregating 15:1	36	38.4		
Segregating 63:1	23	25.6		
Segregating 3:1	21	19.2		
True-breeding white	4	3.2		

PRESTON × 01–24 CROSS.—Kernel-color studies in the F_2 generation in the Preston × 01–24 cross suggested a single-factor difference. The segregation in F_3 substantiated the results of the findings in the previous generation. Three classes were observed in F_3. The proportion of the F_3 rows falling in each of these classes is shown in table 17. The χ^2 test shows a good fit to the expected 1 : 2 : 1 ratio.

TABLE 17.—*Breeding behavior for grain color in the Preston × 01–24 cross and the goodness of fit based on a 1 : 2 : 1 ratio*

Class	Number of progeny		χ^2	P
	Observed	Calculated		
True-breeding white	82	76.7	0.5039	0.7–0.8
Segregating	149	153.4		
True-breeding red	76	76.7		

GLUME COLOR

HOPE × FEDERATION AND PRESTON × 01–24.—The F_2 data on the two crosses, Hope × Federation and Preston × 01–24, indicated that in each case there was a single-factor difference for chaff color. In the Hope × Dicklow No. 3 cross both parents had white chaff, and thus no segregation occurred. Table 18 shows the F_3 breeding behavior of the two crosses for chaff color and the goodness of fit based on a 1 : 2 : 1 ratio. The χ^2 test shows a good fit in both crosses.

TABLE 18.—*Breeding behavior for glume color in the Hope × Federation and Preston × 01–24 crosses and the goodness of fit based on a 1 : 2 : 1 ratio*

Parent	Class	Number of progeny		χ^2	P
		Observed	Calculated		
Hope×Federation	True-breeding white	56	51. 7	0. 7032	0. 7–0. 8
	Segregating	99	103. 4		
	True-breeding bronze	52	51. 7		
Preston×01–24	True-breeding white	76	76. 7	. 4988	. 7–0. 8
	Segregating	149	153. 4		
	True-breeding bronze	82	76. 7		

RELATION OF MORPHOLOGICAL CHARACTERS AND RESISTANCE TO LOOSE SMUT

In order to determine whether a relationship exists between morphological characters and resistance to loose smut, a series of contingency tables was prepared showing a comparison of the reaction to smut infection and morphological characters. A measure of the relationship between the distribution of the two characters being compared may be obtained by calculating χ^2 and determining the value of *P* from Fisher's (*3*) tables. Table 19 shows the distributions and the probability obtained in each case. Table 20 gives a summary of all comparisons. In interpreting results it is safe to assume that if the value of *P* for any given distribution is higher than 0.05, there is no evidence of significant correlation between the characters being considered.

TABLE 19.—*Contingency table for grain color, chaff color, and awns and smut classes as occurring in the* F_3 *progeny of various crosses*

GRAIN COLOR AND SMUT CLASSES IN PRESTON × 01-24 CROSS

Smut (percent)	Number of progeny				χ^2	P
	White	Segregating	Red	Total		
0 to 13.9	59	114	64	237	0. 5489	0. 2–0. 3
14 to 27.9	15	29	16	60		
28 to 41	2	6	2	10		
Total	76	149	82	307		

GRAIN COLOR AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

Smut (percent)	White	Segregating	Red	Total	χ^2	P
0 to 14.9	1	51	82	134	6. 4055	0. 5–0. 7
15 to 29.9	2	15	24	41		
30 to 44.9	1	7	6	14		
45 to 59.9	0	5	8	13		
60 to 74.9	0	2	3	5		
Total	4	80	123	207		

CHAFF COLOR AND SMUT CLASSES IN THE PRESTON × 01-24 CROSS

Smut (percent)	Number of progeny				χ^2	P
	White	Segregating	Bronze	Total		
0 to 13.9	61	116	60	237	2. 1727	0. 7–0. 8
14 to 27.9	13	27	20	60		
28 to 41.9	2	6	2	10		
Total	76	149	82	307		

TABLE 19.—*Contingency table for grain color, chaff color, and awns and smut classes as occurring in the F_3 progeny of various crosses*—Continued

CHAFF COLOR AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

Smut (percent)	Number of progeny: White	Segregating	Bronze	Total	χ^2	P
0 to 14.9	38	65	29	132		
15 to 29.9	9	24	10	43		
30 to 44.9	6	4	4	14		
45 to 59.9	2	5	6	13		
60 to 74.9	1	1	3	5		
Total	56	99	52	207	10.8819	0.2–0.3

AWNS AND SMUT CLASSES IN THE PRESTON × 01-24 CROSS

Smut (percent)	Number of progeny: No. 1	Segregating	No. 4	Total	χ^2	P
0 to 13.9	62	123	52	237		
14 to 27.9	14	29	17	60		
28 to 41.9	2	6	2	10		
Total	78	158	71	307	1.4196	0.8–0.9

AWNS AND SMUT CLASSES IN THE HOPE × DICKLOW NO. 3 CROSS

0 to 12.9	64	113	59	236		
13 to 25.9	13	26	11	50		
26 to 55.0	5	11	5	21		
Total	82	150	75	307	0.4486	0.95–0.98

AWNS AND SMUT CLASSES IN THE HOPE × FEDERATION CROSS

Smut (percent)	Number of progeny: No. 1	No. 2	No. 3	No. 4	Total	χ^2	P
0 to 14.9	7	10	9	10	36		
15 to 29.9	4	2	2	3	11		
30 to 44.9	3	1	3	1	8		
45 to 59.9	1	1	2	1	5		
60 to 71.9	1	0	0	0	1		
Total	16	14	16	15	61	6.9854	0.8–0.9

Since the lowest P value in table 20 is between 0.2 and 0.3, there seems to be no indication of relationship between resistance or susceptibility and any of the morphological characters studied. This is interesting in view of the fact that Fromme (*5*, *6*) has reported that awned varieties were more susceptible to loose smut than were awnless varieties. The question arises, was there any relationship between these two characters from the standpoint of inheritance, or did it just happen that those awned varieties with which Fromme was dealing smutted more than the awnless? The studies herein reported indicate that there is no relationship between awns and susceptibility or resistance; in fact, the awned varieties used in these studies were resistant, and the awnless were susceptible.

TABLE 20.—*Summary of the* χ^2, *and P values as given in table 19*

Cross	Characters tested for relationship	χ^2	*P*
Hope × Federation	Grain color and smut reaction	6.4055	0.5 -0.7
Preston × 01-24	do	.5489	.2 - .3
Preston × 01-24	Chaff color and smut reaction	2.1727	.7 - .8
Hope × Federation	do	10.8819	.2 - .3
Preston × 01-24	Awns and smut reaction	1.4196	.8 - .9
Hope × Dicklow No. 3	do	.4486	.95- .98
Hope × Federation	do	6.9854	.8 - .9

SUMMARY

Genetic studies on the inheritance of loose-smut resistance, awns, grain color, and chaff color are reported for the Hope × Federation, Hope × Dicklow No. 3, and Preston × 01-24 crosses.

The relative effects of the time and method of inoculation as related to infection are also reported. Maximum infection was obtained only when the smut spores were placed directly on the stigmas. There appeared to be little or no difference in the amount of infection occurring when the plants were inoculated at the time the stamens were rather green and immature and when they were inoculated when the plants were in bloom. In the inheritance studies on loose smut, triplicate randomized plantings were made in two of the crosses and duplicate seedings in the other. This permitted a statistical study of the size of the experimental error. The analysis-of-variance method was used in these studies from the error obtained in the various crosses, it was used to determine a difference which might be considered as significant, on the basis of a probablility of 0.05 between two percentages of infection. The amount obtained was used as the class interval in determining the inheritance of resistance to loose smut. The number of F_3 rows falling within the various class intervals, along with the reaction of the parental material to the disease, formed the basis for the proposed factorial relationship.

The genetic studies of awns and both kernel and chaff color were made in the usual way. The proposed genetic composition of the parental material, for the characters studied, based on their behavior in the previously mentioned crosses is given in table 21.

There was no evidence in the studies made of any relationship between the morphological characters and resistance to loose smut.

TABLE 21.—*Proposed genetic composition of parental material for characters studied in crosses made*

Variety	Loose smut	Awns	Kernel color	Glume color
Hope	R_1R_1 R_2R_2 R_3R_3	*AA BB*	K_1K_1 K_2K_2 K_3K_3	*gg*
Preston	R_1R_1 R_2R_2 r_3r_3	*AA BB*	K_1K_1 k_2k_2 k_3k_3	*gg*
01-24	r_1r_1 R_2R_2 R_3R_3	*aa BB*	k_1k_1 k_2k_2 k_3k_3	*GG*
Dicklow No. 3	r_1r_1 r_2r_2 R_3R_3	*aa BB*	k_1k_1 k_2k_2 k_3k_3	*gg*
Federation	r_1r_1 r_2r_2 r_3r_3	*aa bb*	k_1k_1 k_2k_2 k_3k_3	*GG*

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THE MANGANESE CONTENT OF GRASSES AND ALFALFA FROM GRAZED PLOTS[1]

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INTRODUCTION

The nutritive value of manganese and its occurrence in animal and plant tissue has received considerable attention within the last few years. The literature relating to manganese in plant tissue has been reviewed by Lindow and Peterson (*6*)[3], who reported analyses of 84 samples representing the principal classes of human foods. In a later paper by Skinner and Peterson (*12*), analyses of 54 feeding stuffs were given, which show a wide variation in the manganese content of plant material. Several other investigators (*9, pp. 25–26*) have contributed to the knowledge of the occurrence of manganese in foods and feeding stuffs.

In this paper is shown the manganese content of orchard grass (*Dactylis glomerata* L.), domestic rye (*Secale cereale* L.), tall oatgrass (*Arrhenatherum elatius* Beauv.), meadow fescue (*Festuca elatior* L.), timothy (*Phleum pratense* L.), Kentucky bluegrass (*Poa pratensis* L.), redtop (*Agrostis palustris* Huds.), bromegrass (*Bromus inermis*), and alfalfa (*Medicago sativa* L.). The samples used for analysis were collected from the experimental substation plots at Caldwell, Idaho. Just before the plots were grazed, samples from each were taken and sent to the laboratory for analysis. The samples were then dried, ashed, and analyzed as described in the following paragraphs.

METHODS OF ANALYSIS

Since the anhydrous sodium carbonate fusion of the ash in biological material had shown increased recovery of calcium and magnesium (*3, 8*), it was felt that the method might well be applied to the colorimetric determination of manganese. The simplicity of the method and its many advantages in manipulation recommended it as a rapid and accurate means of getting complete recovery of manganese in plant tissue.

One to two grams of the air-dry material to be analyzed was weighed into a platinum dish and ashed overnight in an electric muffle at 600° C. The ash was then fused with 3 g of anhydrous sodium carbonate. After cooling, the fused mass was placed in a 250-cc beaker and covered with a watch glass. Distilled water was added in sufficient quantity to moisten the sample and then 15 cc of 20-percent sulphuric acid (by volume) was added. When the fused material was dissolved, it was washed from the platinum crucible with

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[3] Reference is made by number (italic) to Literature Cited, p. 662.

a few cubic centimeters of 5-percent sulphuric acid. A few drops of 15-percent sodium bisulphite were added to the solution until all the manganese was reduced to manganous sulphate. The solution was then boiled to expel excess sulphur dioxide, filtered, and the acid-insoluble residue washed with several small portions of 5-percent sulphuric acid.

To the filtrate was added approximately 0.3 g of potassium periodate. The beaker was covered with a watch glass and its contents boiled for 5 minutes. The solution was allowed to stand for 1 hour at a temperature of 95° to 100° C. to insure complete oxidation of the manganese. The solution was then diluted to 90 to 95 cc with 5-percent sulphuric acid previously boiled with a little potassium periodate, and cooled to room temperature, the entire sample transferred to a 100-cc colorimetric tube and compared in a colorimeter with a standard manganese solution. A standard solution containing 0.0025 mg of manganese per cubic centimeter was a satisfactory one to use.

The chlorides were not removed from the samples; this step seemed unnecessary because of the small amount that is present in plant material. According to Willard and Greathouse (*14*), the presence of chlorides does not interfere with the final development of color, as the chlorides may be driven off by prolonging the time of oxidation and adding an excess of potassium periodate. Richards (*11*) has shown that excess acidity prevents full development of color or causes it to fade. The acidity, therefore, was kept between 5 and 6 percent. In the large number of samples analyzed no fading of color was observed. It was possible to let the samples stand for several hours and check the first reading, provided they were kept free from the fumes in the laboratory.

The marked agreement in the results of analyses of 1-g samples of the same grass (orchard grass), is shown in the following tabulation:

Sample no.—	*Manganese (milligrams per kilogram)*
1	177. 7
2	177. 7
3	176. 5
4	181. 7
5	178. 7
6	180. 0
7	179. 2
8	179. 2
Average	178. 8

As a further test of the method, known quantities of manganese were added to samples of air-dry timothy hay, and recovery determinations were then made. Table 1 shows that excellent recovery was obtained, considering the fact that a very small quantity of manganese was added as compared with that present in the sample. The difference in recovery of manganese was well within the experimental error of the colorimeter.

TABLE 1.—*Recovery of known quantities of manganese added as manganese sulphate to 2 grams of air-dry timothy hay*

Manganese added (milligram)	Total manganese found	Manganese in grass taken	Added manganese found	Recovery	Manganese added (milligram)	Total manganese found	Manganese in grass taken	Added manganese found	Recovery
	Milligram	*Milligram*	*Milligram*	*Percent*		*Milligram*	*Milligram*	*Milligram*	*Percent*
0.025	0.210	0.1825	0.0275	110	0.030	0.210	0.1825	0.0275	92
	207	1825	0245	98		.212	.1825	.0295	98
	.206	.1825	.0235	94		.215	.1825	0325	108
0.050	232	1825	.0495	99	0.020	.205	1825	.0225	112
	.230	1825	0475	95		200	1825	0175	87
	235	.1825	.0525	105		203	.1825	0205	102
0.015	197	1825	0145	97	0.035	215	.1825	0325	93
	.195	.1825	0125	83		.218	.1825	.0355	101
	195	1825	0125	83		220	.1825	.0375	107

A comparison of the percentage recovery of manganese by the sodium carbonate fusion method, by the hydrofluoric-sulphuric acid digestion method (*1*), and by the official method (*5*) is presented in table 2. The hydrofluoric-sulphuric acid digestion method was used as a base of 100-percent recovery to facilitate the comparison. It will be observed that the results obtained by the anhydrous sodium carbonate fusion method showed very good agreement with those obtained by the hydrofluoric-sulphuric acid digestion method, but the recovery of manganese ranged from 15 to 35 percent lower by the official method than by the other two methods. The sodium carbonate fusion method has been used in a large number of analyses of plant material and has given very satisfactory results.

TABLE 2.—*Comparison of the 3 different methods used for the analysis of manganese in 7 grasses*

Grass	Hydrofluoric-sulfuric acid digestion of plant ash	Sodium carbonate fusion of plant ash	Official method	Sodium carbonate fusion / Hydrofluoric acid digestion	Official method / Hydrofluoric acid digestion
	Mg per kg	*Mg per kg*	*Mg per kg*	*Percent*	*Percent*
Orchard grass	150	150	122	100.0	81.3
Domestic rye	122	120	102	98.3	83.6
Tall oatgrass	110	111	94	100.9	85.4
Meadow fescue	105	107	90	101.9	85.7
Kentucky bluegrass	77	76	64	98.7	83.1
Bromegrass	150	152	110	101.3	73.3
Redtop	230	228	150	99.1	65.2

EXPERIMENTAL PLOTS

The soil used for the experimental plots is a Boise sandy loam. It is described (*4, p. 426*) as a

> . . . . grayish-colored light sandy loam, with a soft, ashy feel, carrying a large amount of silt and having an average depth of about 2 feet. The subsoil of this type south of Boise River is a loam or clay loam which has an average depth of about 18 to 24 inches. This in turn is underlain usually with a sandy loam, but sometimes with sand, generally cemented together with calcium carbonate, forming a hardpan.

In the late summer of 1930 one area of a field was fenced and divided into 9 plots. These plots were about 25 feet wide and 20 rods long, the length of the plots being parallel to the slope of the field. All the plots had uniform drainage, slope, and irrigation. Prior to the experimental work the following crops had been grown on this soil: In 1921, corn; in 1922 and 1923, barley; in 1924 and 1925, wheat; and in 1926 to 1930, alfalfa.

The plots were grazed according to good pasture practice. Every 2 weeks during the grazing season dairy heifers were allowed to graze on the plots for 1 to 3 days. After the stock had been removed, the plots were irrigated, but no fertilizer was applied to any of them during the experiment.

Since the plots were grazed instead of clipped, definite yield data were not obtained. However, the approximate height of each variety of grass was determined at the time the sample was taken for analysis, that is, just before the heifers were admitted to the plots every 2 weeks throughout the grazing season. The fastest growing grass, tall oatgrass, averaged 6.4 inches in height; orchard grass, 3.6 inches, bromegrass, meadow fescue, redtop, timothy, and domestic rye, averaged 2.6 inches. Kentucky bluegrass, the slowest growing grass, averaged 1.9 inches. Alfalfa averaged 5.5 inches. All the grasses, and the alfalfa, grew more rapidly in the early part of the season.

A representative sample of soil from each of the plots was taken during the grazing season and analyzed for manganese and calcium carbonate, and the pH values were determined. The manganese was determined as described in the official methods (*7*). Calcium carbonate was determined by the Puri method (*10*) and the pH values by the hydrogen-electrode method. The soils of all the plots were uniform in manganese and calcium carbonate and in pH values (table 3).

TABLE 3.—*pH and percentage of manganese and calcium carbonate of the soil of 9 pasture plots*

Plot planted in	Manganese	Calcium carbonate	pH	Plot planted in	Manganese	Calcium carbonate	pH
	Percent	*Percent*			*Percent*	*Percent*	
Orchard grass	0.0706	1.00	7.86	Bromegrass	0.0715	0.75	7.90
Domestic rye	0710	75	7.23	Redtop	0712	75	7.86
Tall oatgrass	0735	75	7.85	Alfalfa	0712	75	7.72
Meadow fescue	0703	75	7.65				
Timothy	.0711	75	7.55	Average	07123	777	7.72
Kentucky bluegrass	.0707	75	7.86				

RESULTS OF ANALYSIS

Table 4 presents the analyses of samples of orchard grass, domestic rye, tall oatgrass, meadow fescue, timothy, Kentucky bluegrass, bromegrass, redtop, and alfalfa, taken throughout the grazing season.

TABLE 4.—*Manganese content (milligram per kilogram of grass on oven-dry basis) of 8 varieties of grasses and 1 alfalfa, grown under grazing conditions*

Date taken	Orchard grass	Domestic rye	Tall oatgrass	Meadow fescue	Timothy	Kentucky bluegrass	Bromegrass	Redtop	Alfalfa
1932									
June 6	268	197	105	174	86	69	172	188	61
June 20	240	130	81	127	86	77	172	161	41
July 4	292	145	91	160	97	70	166	161	39
July 18	240	120	90	149	97	75	172	214	49
Aug 1	214	85	90	154	106	107	134	214	40
Aug. 15	170	97	82	184	150	83	134	161	47
Aug. 29	214	97	105	176	130	73	134	177	47
Sept. 11	158	90	121	154	130	75	107	161	47
Sept 26	165	123	126	110	142	80	152	244	48
Oct 11	114	135	113	124	120	72	203	240	47
Average	207.5	121.9	100.4	151.2	114.4	78.1	154.6	192.1	46.6

Wide variations are shown in the manganese content of samples of a single variety of grass taken on different dates. In spite of this, certain grasses showed a relatively higher manganese content than others. Orchard grass had the highest average manganese content of all the grasses. Next in order came redtop, bromegrass, meadow fescue, domestic rye, timothy, tall oatgrass, and Kentucky bluegrass. Alfalfa had a lower manganese content than any of the grasses. Since comparative yield data were lacking, it was impossible to compare the total amount of manganese recovered by alfalfa with the total amount recovered by the grasses. A rapid-growing and high-yielding species like alfalfa, although low in manganese, would show a relatively high total recovery of manganese for the season.

Since the soils of the nine plots showed a uniform manganese content, and since all the plots had received the same treatment, it would seem that the difference in manganese content in the eight varieties of grasses was not due to the difference in manganese content of the soil or to a difference in soil conditions. Moreover, no correlation was found between the rate of growth of the eight grasses and their manganese content, nor between the rate of growth of a single variety of grass and its manganese content. It was concluded, therefore, that the difference in manganese content in the eight grasses was due to the difference in the capacity of the grasses for extracting manganese from the soil.

As a check on the foregoing conclusion the writer calculated, from the field data of Wiggans (*13*), the total amount of manganese recovered by seven of the grasses used in this experiment. Wiggans gives the average yield over a 4-year period of several grasses cut as pasture. His yield data, compiled by Ellenberger, Newlander, and Jones (*2*, *p. 6*), give the yield in pounds of dry matter per acre, as follows: Timothy, 1,454 pounds; redtop, 1,557 pounds; meadow fescue, 1,665 pounds; orchard grass, 1,711 pounds; Kentucky bluegrass, 1,387 pounds; bromegrass, 1,690 pounds; tall oatgrass, 2,044 pounds.

The total amount of manganese recovered for each grass in pounds per acre, based upon the preceding yield data, was as follows: Orchard grass, 0.0355 pound; redtop, 0.0299 pound; bromegrass, 0.0262 pound; meadow fescue, 0.0252 pound; tall oatgrass, 0.0205 pound; timothy, 0.0166 pound; and Kentucky blue grass, 0.0109 pound. With the exception of tall oatgrass, there was a close correlation between the total amount of manganese recovered by the grasses during the grazing season and their manganese content. The exception of tall oatgrass was to be expected, as this was one of the fastest growing and largest yielding grasses.

Since complete information on the manganese requirements of animals, and on the effect of an excess or deficiency of manganese in grasses is not available, it is impossible to determine to what extent the feeding value of the pasture is affected by its manganese content. It is clearly shown from the data presented, however, that the manganese content of pasture may be increased by growing such grasses as orchard grass, redtop, bromegrass, and meadow fescue.

SUMMARY

A new method for the determination of manganese in plant material by the fusion of the plant ash with anhydrous sodium carbonate is presented. This method gives a greater recovery of manganese than is obtained by the official methods.

The manganese content of eight grasses is shown. The average manganese content (dry basis) ranged from 207.5 mg per kilogram for orchard grass to 78.1 mg per kilogram for Kentucky bluegrass. Alfalfa, with an average of 46.6 mg per kilogram was lower in manganese than any of the grasses.

The eight grasses varied markedly in their capacity to extract manganese from the soil.

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PLANT-TISSUE RELATIONS OF THE SUGAR-BEET CURLY-TOP VIRUS[1]

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INTRODUCTION

The distribution of virus in different organs of affected plants received attention from some of the pioneer investigators in the field of plant virus diseases. More recently, consideration has been given to the tissues that may be concerned in the increase and distribution of virus in the plant and to the production of primary and secondary pathologic symptoms. Enough evidence has been accumulated to indicate a wide range of variability among viruses in their relation to various tissues of affected plants.

The virus of true tobacco mosaic furnishes one of the best examples of rapid and extensive invasion of tissues. It seems to have a general systemic distribution in tobacco (*Nicotiana tabacum* L.) and probably invades nearly all the living cells of the plant. Certain other viruses have a more limited distribution and seem able to invade only specific tissues or parts. For example, it is doubtful whether the curl virus of raspberry (*Rubus trigosus* Michx.) occurs in tissue other than phloem, and the virus of the phony disease of peach (*Amygdalus persica* L.) is known to be restricted to the root system in the peach, though distinct pathologic symptoms occur on the tops of affected plants.

Considerable evidence, largely circumstantial, has been accumulated which indicates an intimate relationship between viruses and phloem tissue, and which may be summarized as follows: (1) In virus diseases, such as potato leaf roll and sugar-beet curly top, in which necrotic areas are characteristic, necrosis is largely restricted to the phloem where it begins; (2) in certain virus diseases, notably tobacco mosaic, sugar-beet curly top, and maize streak, the rate of virus spread in the plant is best explained by assuming that the phloem is the main channel of movement; (3) it is believed by some workers that most insect vectors habitually feed on vascular tissue, and this has been shown to be true in the case of the vector of sugarcane mosaic; (4) the virus of raspberry curl and that of two types of raspberry mosaic may be restricted in their movement through the plant by removing rings of bark. Furthermore, in curly top and certain other virus diseases the low percentage of infection obtained by artificial methods of inoculation may be due to inability, with the relatively crude technic available, to place the virus in susceptible vascular tissue without causing injury that inhibits development of the

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virus. Some of the evidence indicates the possibility of complete restriction of virus to the phloem tissue in a few diseases, although it cannot be contended that this may prove to have a general application.

The wide botanical range of plants affected by the curly-top virus offers possibilities for an extensive study of some of the relations of this virus to different plant tissues. Taking advantage of this fact, experiments have been performed with a view to securing data on such problems as that of determining the tissues in which the virus must be placed to produce infection, the tissues on which the vector of the virus of curly top feeds, the tissues from which the virus may be recovered, and the channels and rate of dispersion of the virus in the plant.

ARTIFICIAL INOCULATION

Infection of sugar beets (*Beta vulgaris* L.) or of other plants susceptible to curly top by other means than the feeding of leaf hoppers, *Eutettix tenellus* (Baker), has been obtained with difficulty and in only a very small percentage of the plants inoculated. Severin (*22*)[2] induced infection in beets by making repeated punctures with insect pins into the crown through drops of expressed beet juice. Carsner and Stahl (*8*) were successful in obtaining infection in only a few of a large number of plants inoculated by artificial means. Dana (*11*) in one experiment produced infection in 8 of 16 beet plants. In other trials in which beets, spinach (*Spinacia oleracea* L.), and tomato (*Lycopersicon esculentum* Mill.) were inoculated, a low percentage of infection was obtained.

An effective method of artificial transmission of curly top would facilitate materially the study of many of the problems presented by this disease and its causal agent. The results of artificial methods of inoculation, whether or not successful in producing infection, should throw some light on the general question of the plant tissues in which the virus may multiply and from which it can exert its effects on the plant as a whole. With these points in mind, a number of methods of inoculation were tried, most of which are already in general use.

The plants inoculated included sugar beet, Hubbard squash (*Cucurbita maxima* Duchesne), Turkish tobacco (*Nicotiana tabacum* L.), and Black Valentine bean (*Phaseolus vulgaris* L.). Affected specimens of all these plants and also macerated beet leaf hoppers were used as sources of inoculum. Plants of various ages and conditions of growth were used with the different methods of inoculation. The experiments were made at Riverside, Calif., from 1929 to 1932.

NEGATIVE RESULTS OF INOCULATIONS THROUGH XYLEM

In the earlier experiments attempts were made to infect through the xylem elements of the vascular bundles. Twenty plants having roots approximately 1 inch in diameter were taken from the soil, the lower third of the main root was cut away, and the cut surface of the remaining root was placed in centrifugalized juice from diseased beets. The plants were placed for 8 hours in a dry atmosphere, to increase transpiration, and were then transplanted to 6-inch pots. All remained healthy.

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[2] Reference is made by number (italic) to Literature Cited, p. 700.

In a modification of the above experiment, 10 beets were transplanted to 3-inch pots, a portion of the main root of each beet being allowed to project through the drainage hole at the bottom of the pot. The 3-inch pots were set on the surface of soil in 6-inch pots with the projecting root embedded in the soil of the larger pot. After the plants had become adjusted to this new condition the smaller pot, with the projecting root system, was carefully removed from the 6-inch pot, and the exposed roots were washed free from soil and severely pruned. The 3-inch pots were then placed over containers so that the exposed part of the beet root was immersed in a liquid composed of 1 part juice from diseased beets and 3 parts tap water. The soil in the pots was allowed to become quite dry, and the plants were placed in a dry atmosphere to increase the amount of inoculum taken up by the root system. After 48 hours the plants were removed and transplanted to 6-inch pots. No disease developed in any of these plants.

In a later experiment, 20 rapidly growing beets having a crown diameter of about 1 inch were used. A hole was bored through the crown by means of a small-size cork borer. Glass tubing was inserted to a distance of about one fourth of an inch in each end of this hole. The beets were then joined in series by means of rubber tubing and connected to a liter flask containing centrifugalized juice from diseased beets. A layer of heavy oil was poured over the surface of the beet juice in the flask to reduce oxidation. A gravity flow of beet juice through the system was started, the juice being taken from near the bottom of the flask. The flow of beet juice was regulated to about 20 drops per minute by means of a pinchcock at the distal end of the system. This volume of flow was continued for 48 hours, with a change to fresh beet juice every 12 hours. None of these plants developed signs of curly top.

In the experiments described above it is reasonable to conclude that considerable beet juice was taken up by the tracheae of the inoculated plants and that this juice contained active virus. The failure to produce disease indicates that the curly-top virus does not pass from tracheae into cells or tissues that permit it to become established and to initiate pathologic symptoms.

RESULTS OF VARIOUS METHODS OF INOCULATION

Many plants have been inoculated by other methods. These methods consisted of puncturing leaves, cotyledons, and crowns of young plants through drops of inoculum by means of small needles; rubbing leaves with rolls of cheesecloth saturated with inoculum; and injecting inoculum into the hollow stems of squash and into the pith of tobacco by means of a hypodermic needle.

Inoculum was prepared in the following ways: (1) Diseased plants were ground in a meat chopper, the juice was expressed and centrifugalized, and the relatively clear liquid was decanted and used as inoculum; (2) viruliferous beet leaf hoppers were macerated in a small amount of water in a mortar and used as inoculum; (3) the surfaces of the crowns of diseased beets were cut away with a sharp knife and the exudate from the cut surface was collected and used as inoculum.

The results of these inoculations are given in table 1. Centrifugalized juice from diseased plants proved to be a very poor source of infectious material with the methods of inoculation employed.

Macerated leaf hoppers likewise were a poor source of infectious material, although the number of plants inoculated was too small to justify final conclusions. The best results were obtained from the use of exudate from the cut surface of diseased beets. With this material, 14 of the 124 plants inoculated became infected. Although this is a low percentage of infection, it is so much higher than that obtained by the use of expressed juice that it is worthy of further trial.

HOW THE BEET LEAF HOPPER FEEDS

With few exceptions, insects that are important vectors of plant viruses have mouth parts adapted for sucking plant juices. The feeding habits of a considerable number of species of sucking insects have been studied by several investigators who have determined the relation of feeding punctures to specific tissues. These investigations have dealt predominantly with insects not associated with the spread of plant viruses, though several of the species cause severe injury to their host plants as a result of the introduction of toxic substances.

TABLE 1.—*Results of artificial inoculation of sugar beet, Turkish tobacco, Hubbard squash, and Black Valentine bean with virus from different sources*

Inoculum	Plant inoculated	Method of inoculation [a]	Number of plants	
			Inoculated	Infected
Juice of beet	Beet	1	80	0
Do	Hubbard squash	1, 2	80	0
Do	do	4	20	0
Juice of Hubbard squash	Beet	2	80	0
Do	do	1	80	0
Juice of Turkish tobacco	Turkish tobacco	2	80	0
Do	do	1	20	1
Do	Beet	1	80	0
Juice of Black Valentine bean	Black Valentine bean	3	80	0
Crushed beet leaf hoppers	Beet	1	80	0
Phloem exudate from beet	do	1	124	14

[a] Numbers in this column refer to the following methods of inoculation: 1, Needle punctures into crown through drops of inoculum; 2, needle punctures into crown through diseased leaves; 3, gentle rubbing of leaves with a roll of cheesecloth saturated with inoculum; 4, inoculum injected into the hollow stem of squash or into the pith of tobacco by means of a hypodermic needle.

Büsgen (*6*), Davidson (*12*), Horsfall (*16*), Kenneth M. Smith (*25*), and others have shown that aphids, which constitute by far the most important group of vectors of plant viruses, feed on the phloem tissues of the plants on which they live. Other sucking insects feed on parenchyma or vascular tissue or both, depending on the species. Leaf hoppers as a group, obtain food material from a number of tissues. Smith and Poos (*24*) found that of 6 species studied 5 fed primarily on the mesophyll of the leaf and 1 on the phloem.

In only a few instances have careful studies been made of the feeding habits of insects in relation to transmission of plant viruses. Brandes (*5*) has shown that *Aphis maidis* Fitch, a vector of sugarcane mosaic, makes the phloem its primary objective. The stylets of this insect penetrate the epidermis directly, pass through cells and intercellular spaces of the underlying tissues, and terminate in the phloem of a vascular bundle. Kenneth M. Smith (*26*) states that the species of aphids that transmit potato leaf roll, as well as species that do not transmit this disease, feed on the phloem.

No information has been published regarding the tissues from which the beet leaf hopper obtains its food supply. Observations on leaf hoppers caged on beets indicate that they prefer the veins. This preference is especially noticeable if the leaf hoppers are feeding on petioles. In the sugar-beet petiole there are normally five or more large veins and several smaller ones arranged in an arc beneath the convex surface. The larger veins are deep-seated. The smaller veins vary in this respect, but those in the acute angles of the petioles are always very close to the surface. In feeding, leaf hoppers arrange themselves in greatest numbers along these angles as if seeking the smaller and more superficial veins.

To supplement these observations on the feeding of the beet leaf hopper, more detailed investigations have been undertaken. This work has included a microscopic study of mouth parts inserted in the tissue, and similar studies of the feeding punctures in the beet petiole by means of freehand sections of fresh petioles and by means of embedded and stained material.

In obtaining mouth parts fixed in feeding position, leaf hoppers after being starved overnight were placed on beet petioles and allowed to feed until quiet. They were then subjected to a temperature of about 28° F. for several minutes or until they became inactive. A capillary pipette filled with ether was applied to the posterior end of the abdomen of each insect that remained undisturbed on the petiole. The etherized insects were covered with melted agar to fix them firmly in place. Portions of petiole with the agar-embedded leaf hoppers were killed and fixed in Schaffner's chromo-acetic solution, and sectioned in the usual way. A very slight movement of the leaf hoppers before or during the killing and fixing processes resulted in partial or complete withdrawal of the stylets. Many leaf hoppers were found with stylets exserted from the labium but not inserted in the tissue, or only partly so. However, several leaf hoppers were satisfactorily fixed with the mouth parts apparently in normal relation to the plant tissue.

In further studies leaf hoppers were allowed to feed from 12 to 24 hours on petioles and were then removed. Some of the petioles were sectioned immediately; others were killed, embedded, sectioned, and stained. The line of puncture is quite evident in fresh material as well as in stained sections. In penetrating the tissues the leaf hoppers form a sheath which completely encases the stylets. After the stylets are withdrawn this sheath remains and a definite line through the center marks the position of the stylets. In live petioles the sheath when first laid down is almost colorless but soon takes on a yellowish coloration which clearly differentiates it from the plant tissue. In prepared sections it takes a deep safranine stain with the safranine-Delafield's haematoxylin combination (fig. 1).

A study of leaf-hopper mouth parts in feeding position in conjunction with a study of numerous punctures in fresh and prepared material furnishes a complete picture of the relation of mouth parts to the various tissues of the plant during feeding. These studies have shown clearly that the leaf hopper is able to penetrate cell walls without difficulty (figs. 2 and 3). The line of puncture extends from the epidermis through and between cell walls of the subepidermal layers, frequently to vascular bundles. The path of penetration usually is straight and directed toward a vein. However, the path may be curved and frequently is branched near the tip (fig. 1, *A*). Apparently the stylets can be bent in only one direction at a time, but by partly withdrawing them and inserting them in another direction the leaf hopper is able to explore a considerable area. Some trails curve toward a vascular bundle from an initial direction that would have

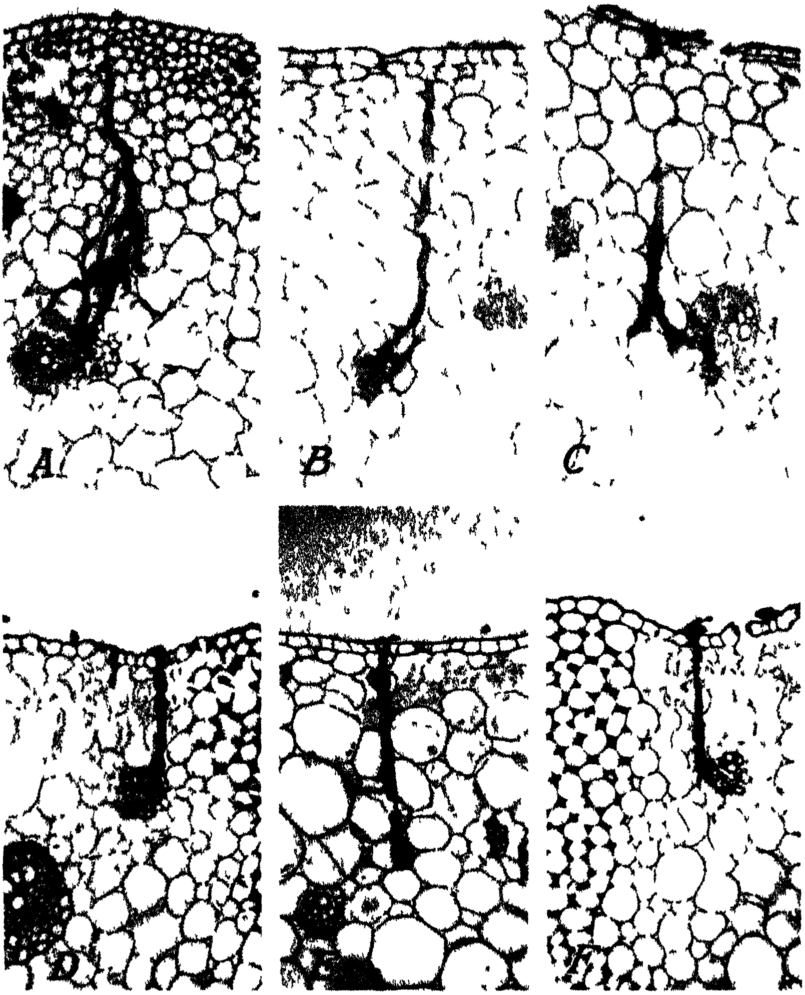

FIGURE 1, *A–F*. Path of feeding punctures of *Eutettix tenellus* in beet petioles. Results of probing are shown in *A* and change of direction to reach vascular bundles in *B* and *C*. A puncture terminating in parenchyma is shown in *F*. × 90

terminated in parenchyma (fig. 1, *C*). Punctures made from the xylem side of the petiole usually veer away from the middle of the bundle and enter the phloem from one side. A few instances of xylem invasion were noted in which copious quantities of exudate were deposited in the tracheae (fig 3). Whether this happened by chance or whether leaf hoppers extract water or food from the xylem is difficult to determine. However, the number of punctures terminating in the phloem and the amount of probing sometimes done, apparently in order to locate the phloem, indicate that this is the tissue of primary importance in supplying food.

One hundred punctures were counted and classified on the basis of the tissue in which they terminated. Of these, 24 terminated in or near the phloem of small veins; 22 terminated in or near the phloem of large veins; 46 terminated in parenchyma outside of the bundles but began from points from which bundles could have been reached.

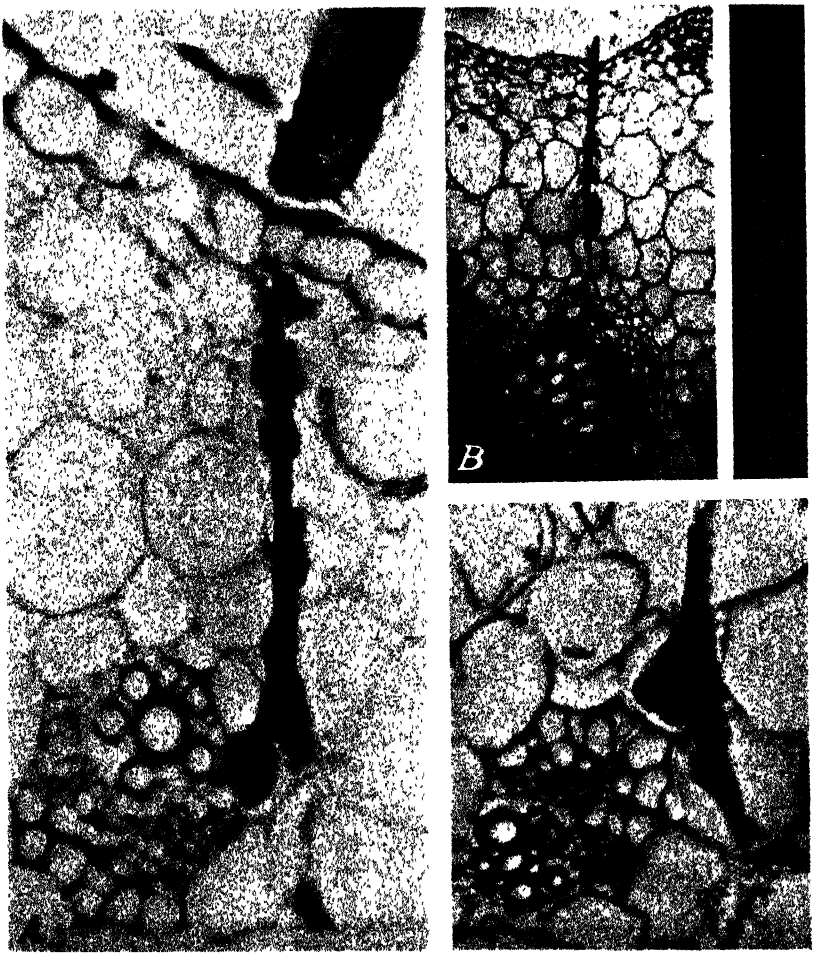

FIGURE 2.—*A–C*, Cross sections of beet petioles. The stylets of *Eutettix tenellus* are embedded in the tissue in the normal feeding position. × 90.

Only 8 trails were found in the parenchyma of the concave side of the petioles originating from points from which bundles could not have been reached. It should be stated that the section from which these counts were made came from small petioles on which large numbers of leaf hoppers had fed. Larger petioles and smaller numbers of leaf hoppers might be expected to give different results and probably would reduce the number of punctures terminating in the parenchyma outside of the bundles, many of which were very shallow and were probably made by leaf hoppers disturbed before maximum penetration had been effected. None of the punctures in the parenchyma was branched nor was there other evidence that the leaf hopper spent much time in exploring such areas. In view of these facts and of evidence to be presented later showing that the leaf hopper derives very little of the life-sustaining materials from parenchyma, it seems probable that in making these punctures the leaf hoppers were merely searching for a more desirable medium from which to extract food.

Several investigators have mentioned the sheath material found in the feeding punctures of sucking insects, but there is a lack of agreement as to whether the sheath material is of plant or of insect origin. Büsgen (*6*) is of the opinion that the sheath laid down by the insects that he studied was composed of material excreted by the insect. Davidson (*12*) considers that the sheath wall of *Aphis rumicis* L. is "composed of substances produced by the reaction of the saliva on the cell sap." Horsfall (*16*) found that the sheath in the feeding punctures left by certain aphids contains proteid material and calcium pectate, and suggests that it is laid down by the plant cells in response to a wound stimulus though the proteid material may possibly be injected by the insect. King and Cook (*17*) suggest that the sheath produced by the sucking insects that they studied results from the action of insect saliva on the middle lamella. F. F. Smith (*23*) has shown that the sheath material in the punctures produced by the potato leaf hopper and the three-cornered alfalfa hopper is largely of insect origin and contains no plant substances with the possible exception of pectose. Brandes (*5*) states that the sheath laid down by *A. maidis* is composed of material given off by the insect. It is obvious that the sheath material formed by *Eutettix tenellus* is composed of material that is given off by the leaf hopper itself. This was demonstrated by a method similar to that described by Fife (*14*), which involved mounting a live leaf hopper under a microscope in such a position as to have the mouth parts inserted horizontally through a membrane into a liquid in the field of vision. In this position the insect may be watched in the process of forming a sheath. Many individuals begin the discharge of a colorless material as soon as the mouth parts penetrate the membrane and continue as the stylets are inserted farther into the medium. The discharge coagulates almost

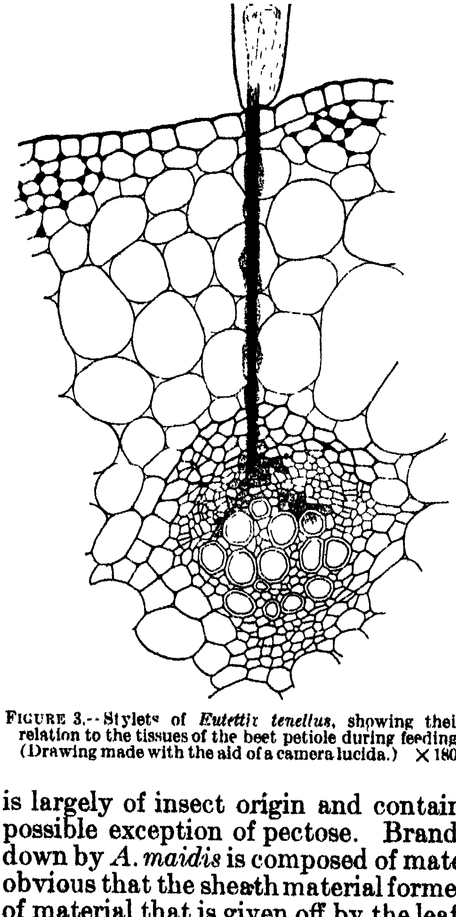

FIGURE 3.—Stylets of *Eutettix tenellus*, showing their relation to the tissues of the beet petiole during feeding. (Drawing made with the aid of a camera lucida.) × 180.

immediately and forms a distinctly visible hyaline sheath around the stylets, which in thickness and general physical properties is similar to the sheath found in freehand and stained sections of the beet petiole. Withdrawal of the stylets leaves a very definite line marking their position. With repeated penetration and partial withdrawal of the stylets a considerable mass of exudate is built up in which stylet trails extending in many directions are visible.

The materials deposited in the plant tissues by nonviruliferous leaf hoppers evidently cause very little injury to the plant as a whole, since a beet plant of average size will support a large leaf-hopper population for a considerable period with no marked ill effect.

Further investigations were made to determine the reaction of individual cells in different types of beet tissue to feeding punctures of nonviruliferous leaf hoppers. Large numbers of leaf hoppers were fed on beet petioles 24 hours and then removed. Microscopic examinations of freehand sections of these petioles were made daily for 10 days and at 5-day intervals thereafter, the last examination being made 20 days after feeding. The sheaths were at first hyaline but soon became yellowish or yellowish brown. They maintained their original relations to the cells to a remarkable degree. By the tenth day, in some instances, the sheath material was displaced in some of the cells and had shrunken slightly. It was still present and easily traced, however, on the twentieth day.

Where collenchyma was traversed, the yellowish color of the sheath was imparted to the thickened parts of the cell walls. This was true also of the cell walls of the bundle cap. Other cells in the path of punctures did not show this change in color of walls. The large parenchyma cells through which the sheaths passed reacted in different ways but all retained their turgidity for 20 days after the punctures were made. Some remained apparently normal, even retaining normal-appearing chloroplasts along with sheath material. Others had a distinctly granular protoplasmic structure and the nucleus in some cases was granular and irregular in outline. The cells were apparently very rarely dead even after 20 days. The cell-wall discoloration in the collenchyma and bundle cap had almost completely disappeared after 15 days, and the vascular bundles seemed normal except for the sheath material remaining in the cells.

Assuming that the phloem is at least the chief reservoir of virus and the place of most rapid multiplication, as stated previously by Brandes (*5*) in the case of *Aphis maidis*, it would be difficult to imagine a mechanism more perfectly designed for virus extraction and introduction than that possessed by the beet leaf hopper. The laying down of a sheath around the mouth parts as they penetrate probably effectually seals off all contents of cells external to the phloem. The introduction of sheath material into the phloem insures the introduction of salivary secretions into this tissue and probably accounts for the introduction of virus. This virus is liberated into a medium rich in nutrients and in a tissue physically adapted for the rapid distribution of inoculum to various parts of the plant, especially to the rapidly growing areas. The same insect mechanism is equally efficient in removing virus directly from the phloem without having the virus come in contact with cell contents of parenchyma tissue or with external agents.

TISSUES FROM WHICH THE LEAF HOPPER OBTAINS FOOD AND EXTRACTS VIRUS

Attempts were made to segregate certain types of beet tissue and to determine their virus content by testing the ability of leaf hoppers to obtain virus from them. These experiments have consisted chiefly of segregating parenchymatous tissues in different parts of the plant and comparing their virus content with adjacent tissues containing vascular bundles. Tissue in which there are no vacsular elements may be isolated from the ventral side of large petioles, from the crown of large beets, from the pith of the flowering stalk, and from seeds in an early stage of development.

However, before accurate conclusions regarding the virus content of tissues from these various sources may be drawn from the results of leaf-hopper transmission experiments, it is necessary to know the effect of the tissues on the leaf hopper and the relative amounts of materials that the leaf hopper is able to extract from them. To throw some light on this problem, the mortality of the leaf hoppers having access to parenchyma tissue from each of the sources just mentioned has been compared with the mortality of leaf hoppers having access to adjacent tissue containing vascular elements. The average length of life of leaf hoppers on the different tissues is taken as a rough relative measure of the food and other materials obtained.

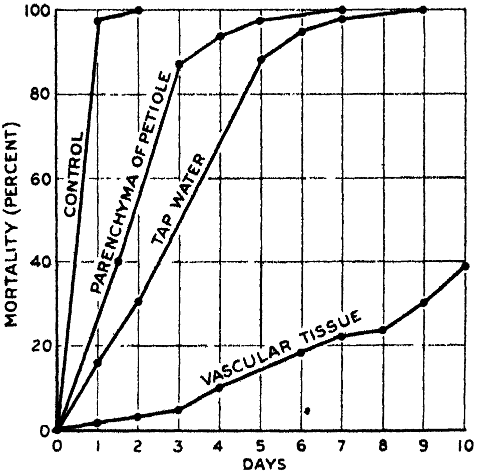

FIGURE 4.—Mortality of *Eutettix tenellus* on different types of beet tissue at 65° to 75° F.

LEAF-HOPPER MORTALITY ON DIFFERENT TYPES OF BEET TISSUE

Mortality tests were made in two separate experiments. In the first experiment, mortality of leaf hoppers having access to vascular tissue of petioles was compared with that of an equal number of leaf hoppers having access to parenchyma of the ventral side of petioles. The experiment was started with 10 petioles for each type of feeding, and 10 leaf hoppers were placed on each petiole. Fresh petioles were supplied every 48 hours. The petioles were covered with a thick coating of paraffin, with strips of paraffin about one fourth of an inch wide and 3 inches long removed to expose parenchyma tissue on the concave side in one series and vascular tissue along the acute angles of the petioles in another series. As a further check on these treatments, a third lot of 100 leaf hoppers was placed in small cages where they had access to tap water through a parchment membrane, and a fourth lot of 100 was placed in small cages without food or water. The experiment was run at relatively low temperatures (60°–75° F.) and discontinued at the end of 10 days. The results are shown graphically in figure

All leaf hoppers receiving neither food nor water were dead at the end of the second day. The mortality curve of the lot receiving parenchyma is roughly parallel with that of the lot receiving tap water, although the death rate is slightly lower in the latter group; mortality reached 100 percent on the seventh and ninth days, respectively. The leaf hoppers having access to the vascular tissue thrived much better, only 39 percent being dead at the end of the tenth day.

In a second experiment, tissue from additional sources was used and the leaf hoppers were kept at a temperature of 90° to 100° F. The different lots of insects in materials available for this experiment were given, respectively, (1) neither food nor water, (2) tap water, (3) parenchyma of the petiole, (4) vascular tissue of the petiole, (5) young seeds, (6) hull of the seed ball, (7) pith from the crown and flowering stem, and (8) tissue containing vascular elements, from areas adjacent to the pith of the flowering stalk and the crown. The experiment was run in duplicate series, 50 leaf hoppers being used in each treatment in each series. The test was discontinued at the end of 48 hours. The results are shown graphically in figure 5.

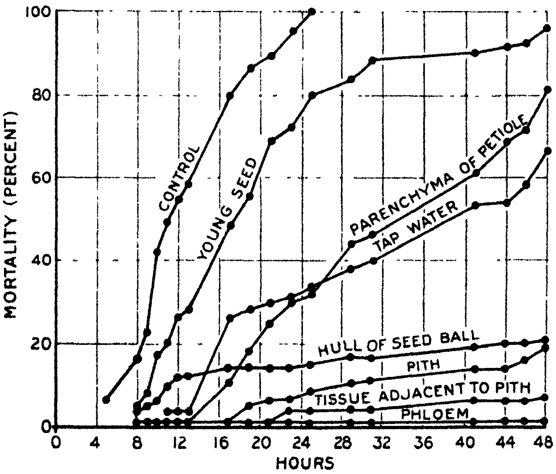

Figure 5.—Mortality of *Eutettix tenellus* on different types of beet tissue at 90° to 100° F.

As measured by the control treatment in which the leaf hoppers received neither food nor water, each of the types of tissue on which the insects were allowed to feed yielded some life-sustaining materials. Young seeds proved to be poorest in this respect, probably in part because of their tendency to dry very rapidly. The mortality curve, however, is steep for parenchyma from all sources except the flowering stalk and crown.

In general, these experiments indicate that parenchyma is unfavorable for the beet leaf hopper. Parenchyma of the petiole seems to be little better than tap water. Parenchyma of the flowering stalk and crown is more favorable and ranks in food value next to tissue containing vascular elements. It is worthy of note that parenchyma from the flowering stalk and crown is higher in sugar than parenchyma from other sources, and it is possible that this greater sugar content is responsible for the prolonged life of the leaf hoppers. Normally, of these types of parenchyma, only that from the petiole is available to leaf hoppers. These experiments furnish additional evidence to support the view that the beet leaf hopper is chiefly dependent on the phloem for its food.

The next question of importance is whether the leaf hopper feeds sufficiently on parenchyma from the various sources to acquire virus if it is present. Experiments have indicated that a relatively short period of feeding is sufficient for leaf hoppers to acquire virus from diseased beet leaves. A few individuals have become viruliferous after a 1-minute feeding period, and larger numbers acquire virus as the feeding period is increased. Of 150 leaf hoppers fed singly on diseased beet leaves, 11 percent acquired the virus in 5 minutes. In another test with the same number of leaf hoppers, 23 percent became viruliferous after a 10-minute feeding. Since the evidence shows that the beet leaf hopper acquires enough material from parenchyma to appreciably prolong its life, and since the leaf hopper acquires the virus in a very short period of feeding on diseased beet leaves, it seems reasonable to assume that the leaf hopper may be used to furnish evidence regarding the presence or absence of virus in parenchymatous tissue despite the fact that parenchyma is not a very favorable source of food.

VIRUS CONTENT OF TISSUES OF BEET PLANT

Assuming that the method just described affords a means of testing for the presence of virus, nonviruliferous leaf hoppers have been given access to various types of tissue isolated from diseased beets. At the time the leaf hoppers were given access to parenchyma a second group of leaf hoppers was placed in an empty cage to serve as a check on the feeding of the leaf hoppers on parenchyma. When all the leaf hoppers serving as checks were dead the insects surviving on parenchyma were transferred to seedling plants. These checks were used in all tests except those involving the parenchyma of the petiole.

Parenchyma of Petiole

Large petioles from beets infected during the current season were covered with paraffin, and strips of this were removed to expose parenchyma or vascular tissue, as described previously. Forty nonviruliferous leaf hoppers were allowed to feed on each petiole, 20 for 24 hours on parenchyma and 20 for 24 hours on vascular tissue. One half of the petioles had parenchyma exposed during the first 24 hours and vascular elements exposed during the second 24 hours, and the other half had the exposures made in reverse order. At the end of the feeding period the leaf hoppers were divided into lots of 5 each and caged on healthy plants. In this manner 8 healthy sugar-beet plants were inoculated from each petiole; 4 plants by means of leaf hoppers which had access to parenchyma and 4 by means of leaf hoppers which had access to vascular elements. One hundred and four plants were inoculated by means of leaf hoppers from each type of tissue. Of the 104 plants inoculated by means of leaf hoppers from vascular tissue, 42 became infected; whereas, of the 104 plants inoculated by means of leaf hoppers from parenchyma, only 1 became infected. The results of this experiment are shown in table 2.

In a second experiment, petioles were taken from plants that had been diseased several months and on which petioles had been produced subsequent to infection. The plan of this experiment was the same as that just described, except that larger numbers of petioles were used and only one lot of leaf hoppers was given access to each petiole. In this test, of the 80 plants inoculated by means of leaf hoppers from tissue containing vascular elements, 37 became infected; whereas, of the 80 plants inoculated by means of leaf hoppers from parenchyma tissue, none showed any sign of disease (table 2).

TABLE 2.—*Virus content of sugar-beet tissues as indicated by leaf-hopper tests*

Tissue tested	Leaf hoppers fed	Plants inoculated [a]	Plants infected	
	Number	*Number*	*Number*	*Per cent*
Vascular tissue, petiole of first-year beets	520	104	42	40.3
Parenchyma tissue, petiole of first-year beets	520	104	1	.9
Vascular tissue, petiole of second-year beets	400	80	37	46.2
Parenchyma tissue, petiole of second-year beets	400	80	0	.0
Vascular tissue, flowering stalk	420	84	62	73.8
Pith of flowering stalk	420	84	0	.0
Vascular tissue below crown	600	120	58	48.3
Parenchyma tissue below crown	600	120	9	7.5
Outer hull of seed ball	200	40	11	27.5
Young seeds	200	40	0	0

[a] 5 leaf hoppers were placed on each plant inoculated.

PARENCHYMA OF CROWN

Tissue selected as containing no vascular elements was taken from the crown of large diseased beets and placed in a cage containing nonviruliferous leaf hoppers that had been starved overnight. A second lot of tissue containing vascular elements was taken from the portion of the beet adjacent to the first selection and exposed to a second lot of leaf hoppers. After the leaf hoppers had been allowed a feeding period of 5 hours they were divided into lots of 5 each and placed on healthy beet seedlings. One hundred and twenty plants were inoculated by means of leaf hoppers from each of the two food sources. In this experiment, of the 120 plants inoculated by means of leaf hoppers from tissue containing vascular elements, 58 became infected; and of the 120 plants inoculated by means of leaf hoppers from tissue containing no vascular elements, 9 became infected.

In the foregoing experiment, tissue was taken from six beets and the tissue from each beet was used as a separate test. For the six beets the number of infections resulting from the leaf hoppers that had access to parenchyma tissue was, respectively, 0, 1, 5, 0, 3, and 0. The infections resulting from leaf hoppers that had fed on adjacent vascular tissue from the same beets were, respectively, 6, 9, 18, 16, 9, and 5. Each value represents the number of infections obtained from inoculating 20 plants.

These results seem to demonstrate that virus does occur in some types of parenchyma tissue. Perhaps if virus occurs in any parenchyma tissue of the plant it would be expected to be present in the parenchyma that lies immediately below the growing point and closest to the actively growing areas of the beet crown. However, even there it seems to occur in diminished concentrations—in some cases in concentrations too low for relatively large numbers of leaf hoppers to pick it up.

PITH OF FLOWERING STALK

Large fruiting stalks were selected from plants that had been infected the season prior to flowering. Portions of pith containing no vascular tissue were removed and exposed to the feeding of nonviruliferous leaf hoppers. A second group of nonviruliferous leaf hoppers was allowed to feed on tissue containing vascular elements selected from the area immediately adjacent to the pith that was used as food for the first lot. Eighty-four plants were inoculated from each lot of leaf hoppers. Of the 84 plants inoculated by means of the leaf hoppers from tissue containing vascular elements, 62 became infected; whereas of the 84 plants inoculated by means of the leaf hoppers from pith, none showed any sign of disease.

YOUNG SEEDS

Severin (*21*) has shown that there is no seed transmission of the curly-top virus in beet. Since this is true, the question arises as to whether the virus never gains access to the seed in any stage of its development or whether it may be present in certain early stages of seed development and become inactivated as the seed matures.

To obtain information as to whether virus occurs in seeds in the earlier stages of their development, young seeds from diseased plants were separated from the surrounding tissue and placed in cages where nonviruliferous leaf hoppers had access to them. Very young seeds having a high water content were selected. As a check on the virus content of the nearby tissue, the hulls of the seed balls from which the seeds were removed were placed in cages with other nonviruliferous leaf hoppers. After periods of several hours the leaf hoppers were divided into groups of five each and placed on seedling beets. Of the 40 plants inoculated by means of leaf hoppers from hulls, 11 showed signs of the disease; whereas of the 40 plants inoculated by means of leaf hoppers from seeds, none became infected.

Since no virus was obtained from the seeds by the leaf hoppers, it seems probable that seeds contain no virus even in the early stages of their development and that virus may not be able to pass from the plant into the seed. Therefore, absence of seed transmission may be due to a barrier between the embryo and the mother plant which, although permitting passage of water, mineral elements, and elaborated foods, restrains or inactivates the virus.

CONCENTRATION OF VIRUS IN PHLOEM EXUDATE

The foregoing experiments show that leaf hoppers readily acquire virus from vascular tissue and rarely obtain it from other tissues. Since the xylem elements evidently do not carry any considerable amount of virus, the phloem must contain at least the greater part of the virus in the vascular elements. Liquid from phloem tissue may be obtained by making cuts across the tops of beet roots. In a few minutes drops of exudate appear above the severed ends of vascular bundles and may be collected with capillary tubes and used in artificial feeding tests with leaf hoppers. Attempts were made to compare the relative virus concentration of such exudate with that of the expressed juice from the entire beet. In these tests, drops of exudate from diseased beets were placed on a parchment membrane. Nonviruliferous leaf hoppers were allowed to feed on the exudate through the membrane for about 4 hours and then were caged singly on seedling beets.

A second lot of nonviruliferous leaf hoppers were allowed to feed on expressed beet juice and then were caged singly on seedling beets. The results shown in table 3 indicate that more virus is available to the leaf hopper from phloem exudate than is available from expressed juice from the entire beet. Of the 104 leaf hoppers fed on phloem exudate, 33 produced infection; whereas of the 104 leaf hoppers having access to expressed beet juice, only 4 gave evidence of having acquired virus.

TABLE 3.—*Virus content of phloem exudate and expressed beet juice as indicated by leaf-hopper tests*

Material tested	Plants inoculated [a]	Plants infected	
	Number	*Number*	*Percent*
Exudate from phloem of beet root	104	33	31.7
Expressed juice from beet root	104	4	3.8
Exudate from beet petioles	24	7	29.1
Expressed juice from beet petioles	24	0	.0

[a] 1 leaf hopper was placed on each plant inoculated.

It is noted frequently that drops of exudate collect on the leaves and petioles of rapidly growing beets that have been recently infected. This exudate has long been considered to come from the phloem. Recently, Esau (*13*) has made histological studies of diseased beets and described the path which this exudate takes in moving from the phloem to the exterior. By means of exudate of this type further feeding tests were made to determine virus concentration. These tests were carried out as already described, 24 leaf hoppers being given access to exudate and an equal number having access to expressed juice from beet petioles. In this test, 7 leaf hoppers acquired the virus from exudate, whereas none was found to be viruliferous after feeding on expressed juice.

In connection with the experiments that indicate a very low concentration of virus in types of tissue other than phloem, these tests with phloem exudate seem to demonstrate conclusively that the chief virus reservoir in the sugar beet is phloem tissue. Phloem exudate with its high virus content may prove valuable in virus purification experiments and in work dealing with properties of the virus.

MOVEMENT OF VIRUS IN DIFFERENT TISSUES

GRAFT UNIONS OF SUGAR BEET

Twenty healthy sugar-beet plants having main roots approximately three fourths of an inch in diameter were taken from the soil, and a portion of one side of each root was removed by means of a sharp knife. Twenty diseased beet plants were treated in a similar manner. The cut surface of each diseased beet was placed in contact with the cut surface of a healthy beet and the two plants firmly bound together and potted. Symptoms of curly top began to appear on the new leaves of the inoculated beets in 3 weeks. Of the 20 healthy beets, 17 became diseased. In the case of the 3 beets that did not develop symptoms, the diseased member of the pair died probably before union was complete.

GRAFT UNIONS OF TOBACCO

Experiments were conducted with Turkish tobacco to determine at what stage in the development of a graft union the curly-top virus passes from a diseased scion to a healthy stock. Healthy tobacco plants were cut back to a height of about 8 inches, and 3 inches of stem from a diseased plant was grafted to each healthy plant. Cuts were made at angles that afforded considerable surface contact. Grafts were removed from stocks at 24-hour intervals for 15 days and the results on the inoculated plants noted (table 4).

TABLE 4.—*Time required for infection of healthy tobacco stocks by scions from diseased plants*

Period between grafting and removal of diseased scion (days)	Plants grafted	Plants infected		Period between grafting and removal of diseased scion (days)	Plants grafted	Plants infected	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
1	10	0	0	9	12	7	58
2	10	0	0	10	10	7	70
3	10	0	0	11	10	8	80
4	10	0	0	12	10	10	100
5	10	0	0	13	10	9	90
6	13	0	0	14	10	10	100
7	11	3	27	15	10	9	90
8	12	5	41				

No infection occurred until the seventh day. The number of infected plants increased from 27 percent on the seventh day to 100 percent on the twelfth day. The union between the stock and the scion was examined for all the time intervals used. At the end of 3 days a definite union was found which was complete enough to make necessary the use of an appreciable amount of force in removing the scion. Graft unions of different ages from 3 to 9 days were killed, embedded, and sectioned, and then were examined under a compound microscope. In all the specimens sectioned, the union at the end of the third day was composed wholly of meristematic tissue. This rapidly differentiated into other tissues and in the 5- and 6-day-old unions the beginnings of tracheal elements were clearly evident. In the 7-, 8-, and 9-day-old unions, apparently mature tracheal elements with pits and rings were distinctly visible. The walls of these elements were lignified, as indicated by their taking a deep safranine stain. Phloem elements could not be clearly identified, but strands of elongated cells paralleling the tracheae were observed, which may well have included functional phloem.

The foregoing experiment seems to demonstrate that in tobacco infection does not result from contact of cut surfaces and that virus does not move through newly formed meristem in a period of from 2 to 4 days. Infection apparently does not occur until after tracheal elements, and probably phloem elements, connect the stock with the scion. Since the virus seems unable to pass out of tracheae into other cells and become established sufficiently to produce disease, and since it also seems unable to pass through meristem or young parenchyma, it appears extremely probable that in tobacco grafts the virus moves in the phloem in crossing a graft union.

REMOVAL OF RINGS OF BARK[3] AND INTERNAL PHLOEM

Killing portions of stems and removing rings of bark have been the methods used by several investigators to obtain evidence regarding the tissues in which virus is dispersed through plants.

The writer (*2*, *3*) found that the virus causing curl of raspberry and two viruses causing mosaic of raspberry may be limited in their movement through the plant by removing rings of bark.

In tomato, according to Caldwell (*7*), the virus of mosaic bridged an area on the stem from which a ring of bark was removed. As

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"bark" as used in this paper signifies all tissue of the stem from epidermis to cambium, Caldwell points out, however, the tomato has a weak development of internal phloem, so that it is not clear whether the virus passed through the woody cylinder, the pith, or the internal phloem. The same investigator conducted experiments to determine whether the virus moved through dead portions of stem. Of the 26 plants having portions of the stems treated with chloroform, the virus in 14 did not cross the treated area. In 12 plants, virus was present in noninoculated parts. These exceptions are attributed to incomplete killing of the stem, regeneration of tissue, and accidental infection. In plants having portions of the stems killed by steaming, the virus was held in the inoculated parts for several weeks in 20 of the 21 plants used. The one exception was thought to be due to accidental infection and not to movement of virus across the steamed area.

The virus causing curly top has certain characteristics that render it convenient for experimental use in tests involving tissues through which movement occurs. One of the most important of these is its failure to produce infection except as inoculated into plants by its specific vector, thus reducing to a minimum the chance infection of noninoculated parts of plants kept for long periods of time. Moreover, the virus causes disease in a large number of plant species, thus making available a wide range of anatomical types for experimental use.

Since the sugar beet cannot be employed in ringing experiments because of its anatomical structure, it was necessary to choose some other susceptible plant for such experiments. In making a survey of susceptible plants of a more or less woody nature, tests were made on two species of tobacco, namely, common tobacco (*Nicotiana tabacum* L.), Turkish variety, and tree tobacco (*N. glauca* Graham). These plants grow well under a wide range of greenhouse conditions, are easily propagated at all seasons from seeds or cuttings, and are very satisfactory for experiments involving grafting. Moreover, the presence of an internal phloem makes these species suitable for experiments on the movement of virus in the phloem not possible in most other types of woody plants. Each of these species has been used in a fairly extensive series of ringing experiments.

NICOTIANA TABACUM

Turkish tobacco has only a medium degree of susceptibility to infection by the curly-top virus, but symptoms of disease are characteristic and well marked. Infection may be induced by leaf hoppers or by grafting. In most of the experiments about to be described leaf hoppers were used in making inoculations.

The first experiments with Turkish tobacco were planned to determine whether the virus would pass downward through a killed portion of stem. Plants approximately 2 feet tall were cut back to a height of about 10 inches and the buds in the leaf axils allowed to grow to a length of 1 to 2 inches. A portion of the stem below the second or third bud from the top was incased in a celluloid cylinder, the bottom being closed around the stem. Hot paraffin was poured into the cylinder. This killed the tissues and protected and supported the killed area. After a portion of the stem had been killed in this manner, leaf hoppers were allowed to feed on the top bud. Distinct symptoms of curly top appeared in an average period of 7 days on all the inoculated buds of the 10 plants used. The inoculated parts lived for an average period of 16 days. All plants were held for several weeks, but curly-top symptoms appeared in no case in any part of the plant below the killed area.

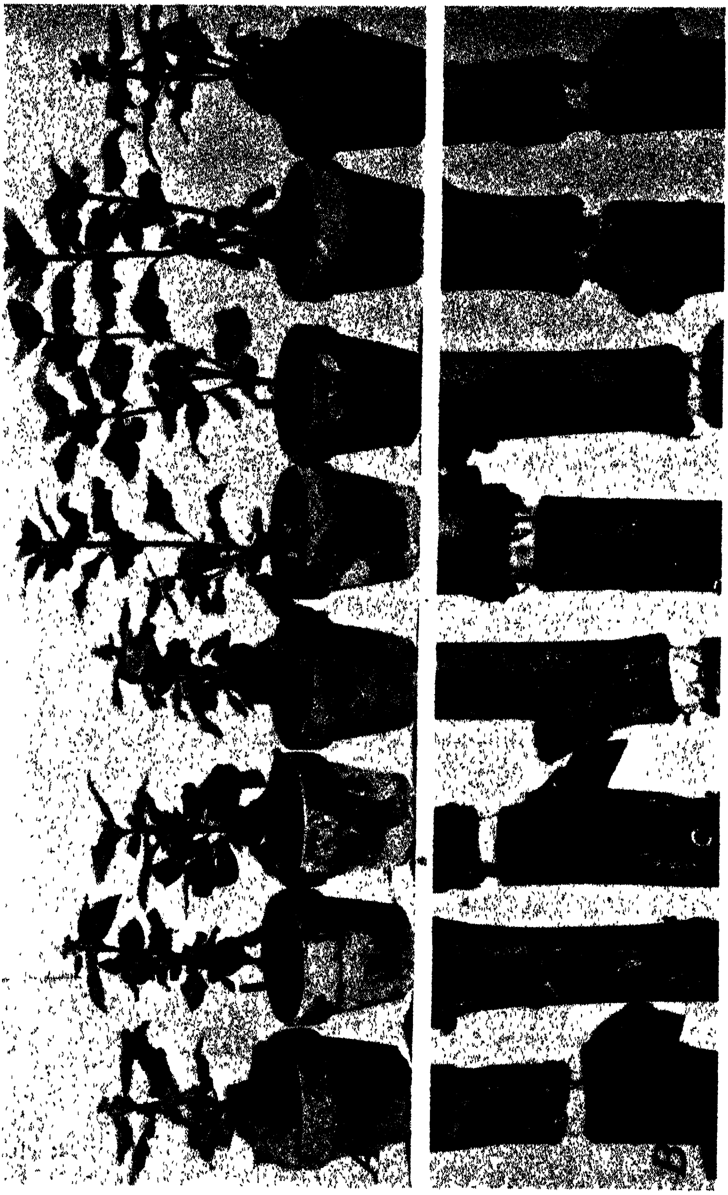

FIGURE 6.—*Nicotiana tabacum* plants after ringing. *A*, Series of plants showing results of interference with virus movement by certain types of rings. The virus moved past the rings in plants *a*, *b*, *c*, *d*, and *h*, and failed to move past the rings in plants *e*, *f*, and *g*, as shown by symptoms and lack of symptoms, respectively, on the shoots coming from below the rings. *B*, *a–h*, Ringed areas of plants shown in *A*. (Plants photographed 1 month after inoculation.)

Further experiments were made to determine the part of the stem through which the virus is able to move. Plants were grown to the beginning of flower-bud production. The stems were pruned back to a height of 18 inches and buds were allowed to start. The plants were then divided into 8 lots and treated as follows:

Lot 1.—A ring of bark was removed from the internode below the second or third bud from the top. This is termed an external ring (fig. 6, *B, a*).

Lot 2.—A hole was made through the bark and wood of the internode about one half of an inch below the second or third bud, by means of a small cork borer and the pith and internal phloem were removed, exposing a ring of wood one fourth of an inch wide on the inner side of the woody cylinder. This is termed an internal ring (fig. 6, *B, b*).

Lot 3.—An outer and inner ring 1 inch apart were made with the outer ring above the inner and the two rings separated by the second or third bud from the top of the plant (fig. 6, *B, c*).

Lot 4.—The plants were treated as in lot 3, except that the relative positions of the two rings were reversed (fig. 6, *B, d*).

Lot 5.—An outer and an inner ring, 1 inch apart, were made with the outer ring above the inner and both rings in the second or third internode (fig. 6, *B, e*).

Lot 6.—The plants were treated as in lot 5 except that the relative positions of the rings were reversed (fig. 6, *B, f*).

Lot 7.—Outer and inner rings were made in the second or third internode, the two rings being placed at the same level on the stem (fig. 6, *B, g*).

Lot 8.—Plants were treated as in lot 7, except that a small strand of bark was left in the outer ring (fig. 6, *B, h*).

These treatments were designed to determine whether the curly-top virus moves (1) through internal phloem, (2) through the external phloem, (3) from the internal phloem to the external phloem through unions of the two in the leaf traces, (4) from the external to the internal phloem through unions of the two in the leaf traces, (5) from the internal phloem to the external phloem through the medullary rays or other parts of the woody cylinder, (6) from the external phloem to the internal phloem through the medullary rays or other parts of the woody cylinder, (7) downward through the woody cylinder, or (8) through a very small strand of bark bridging an external ring.

Immediately after ringing, the top bud on each plant was exposed to viruliferous leaf hoppers. Symptoms of curly top appeared on the inoculated bud in from 6 to 13 days. Typical results of a series of tests are shown in figure 6, *A*, and results of all tests are given in table 5.

Table 5.—*Influence of rings on virus movement in plants of Turkish tobacco (Nicotiana tabacum) cut back to a height of 18 inches, inoculated in top two buds, and ringed 1 to 3 inches below the point of inoculation*

Number and position of rings	Plants infected	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period between inoculation and appearance of symptoms	Plants affected	Average period between inoculation and death of parts above rings
	Number	*Number*	*Days*	*Number*	*Days*
Outer ring only	15	15	9.9	0	
Inner ring only	8	8	11.0	0	
Outer ring 1 inch above inner, bud between	15	15	9.6	0	
Outer ring 1 inch below inner, bud between	15	15	11.1	0	
Outer ring 1 inch above inner, both in internode	11	4	35.0	7	83.8
Outer ring 1 inch below inner, both in internode	16	3	27.0	13	97.5
Outer and inner rings at same level	14	0		14	86.9
Outer and inner rings at same level, strip of bark in outer ring	7	7	10.4	0	

In all cases where there was an uninterrupted channel through either internal or external phloem across the rings the virus moved downward with little apparent delay. In many instances in some types of ringing, symptoms were evident on shoots below the rings before any sign of disease appeared on the inoculated parts. In the type of ringing in which the appearance of virus below the ring was dependent on its movement through a distance of less than one fourth of an inch of woody cylinder, no symptoms appeared in any parts below the rings in any of the 14 plants inoculated. The diseased parts above the rings lived for an average period of 86.9 days from the time of inoculation. In the series of tests in which the two rings were at different levels in the internode and the external ring was the higher, the virus was held in the inoculated parts in 7 of the 11 plants used. The inoculated parts in these 7 plants lived an average period of 83.8 days. In four plants symptoms appeared below the rings in an average period of 35 days. In the series in which both rings were at different levels in an internode and the internal ring was the higher, the virus was held in the inoculated parts in 13 of the 16 plants used, the average life of tops of the 13 plants being 97.5 days. In three plants symptoms appeared in parts below the rings in an average period of 27 days. In series in which there was an uninterrupted path of phloem across the symptoms appeared on the parts below rings in an average of 9.6 to 11.1 days, which differed in different series.

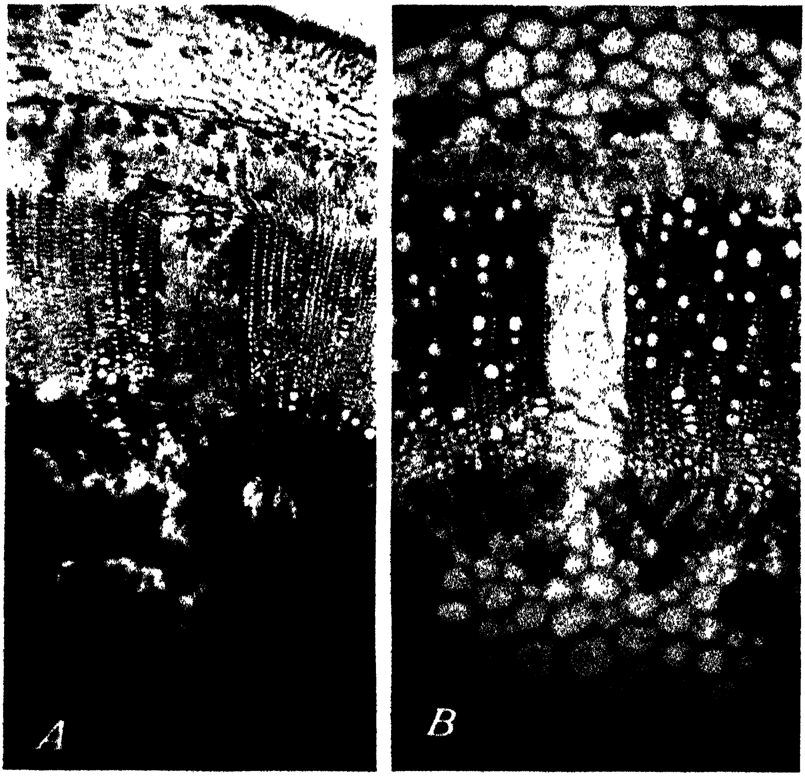

FIGURE 7.—Regeneration of cells in the woody cylinder of tobacco plants having two rings 1 inch apart in an internode: *A*, *Nicotiana tabacum*; *B*, *N. glauca*. × 40.

The seven plants in which symptoms appeared below rings in the two series in which the rings were at different levels in an internode were examined to determine what conditions were present or what changes had occurred that might have a bearing on an explanation of virus passage. Serial sections of entire internodes revealed definite regions of regeneration of what appeared to be medullary rays in one or more areas of the woody cylinder. These regions were sufficiently evident to be recognizable to the unaided eye and seemed to consist of masses of newly formed tissue extending from a region of very active internal phloem outward and downward to the bark, splitting the wood in their growth. These interphloem strands seemed to have their origin just above the top of the internal ring. It is considered probable that the internal phloem transports considerable food under normal conditions but that since the internal phloem is enclosed by a rigid tissue the opportunity for the expression of growth impulses may be limited, and the tendency to initiate regeneration of medullary rays may be greater than from the opposite side of the woody cylinder, where the food brought down in the external phloem is used to form a great amount of callus, wood, bark, and roots. A typical regeneration area is shown in figure 7, *A*. These areas of regenerated tissue were examined microscopically for phloem elements. They were found to be composed of a large variety of active cells of different shapes and sizes, some narrow and very much elongated, others broader but rectangular, and many oval or irregular. Phloem tissue could not be identified definitely, but it seems quite probable under the circumstances that tissue capable of translocating elaborated foods was present in these connecting strands.

NICOTIANA GLAUCA

To have available a more woody stem, a perennial species of tobacco, *Nicotiana glauca*, was chosen for several series of ringing experiments similar to those described for *N. tabacum*. *N. glauca*, however, has not been reported as susceptible to curly top, and before the ringing experiments were started preliminary tests were made to determine the reaction of the species to the curly-top virus. To test susceptibility to infection, large numbers of viruliferous leaf hoppers were allowed to feed on five small plants for several days. None of these plants developed symptoms of curly top. Larger plants, 3 feet or more in height, were then used. These were pruned to a height of about 18 inches, and a 3-inch length of stem from a plant of *N. tabacum* affected with curly top was grafted at the top. The *N. tabacum* scions grew well and developed typical curly-top symptoms. No signs of disease appeared, however, on any of the new growth from the stock. Parts of stems from healthy *N. tabacum* plants were then grafted in at the base of the *N. glauca* stems at points about 14 inches below the diseased scion. These grafts without exception became diseased, showing that the *N. glauca* plants were infected and that the virus had moved downward through approximately 14 inches of stem.

It was later proved that *Nicotiana glauca* is susceptible to infection by direct feeding of leaf hoppers. Healthy *N. tabacum* stems were grafted into the base of *N. glauca* plants and viruliferous leaf hoppers were allowed to feed on young shoots of the *N. glauca* stems. The virus passed from the inoculated shoots through the stem and infected the graft several inches below.

Tests were next made to determine how long the virus would remain active in *Nicotiana glauca*. Two plants which had been inoculated from *N. tabacum* by the grafting method were selected and all *N. tabacum* tissue removed. These two plants were tested at intervals over a long period by grafting portions of their stems on healthy *N. tabacum* plants. The production of disease in this latter species showed that the virus was active in *N. glauca* 2 years after the plants were infected. At no time, however, were signs of curly top visible on these plants. The species seems to be a symptomless carrier of the virus under the conditions of these tests.

The stem of *Nicotiana glauca* is very well adapted to ringing experiments, but since the plant does not show symptoms of the presence of virus it was necessary to modify the technic used in previous experiments with *N. tabacum*. A suitable modification was accomplished by grafting *N. tabacum* on *N. glauca* stems as follows: *N. glauca* plants were grown in 12-inch pots to a height of 3 to 7 feet and pruned to a height of 18 inches. Plants were inoculated by grafting infected *N. tabacum* stems at the top. A second portion of stems from *N. tabacum*, this part from a healthy plant, was grafted in at the base about 14 inches below the top or diseased graft. This latter graft was used as an indicator of the presence of virus in the basal portions of the *N. glauca* plants, and the *N. glauca* stem was used as a medium in which to study the influence of rings in the downward movement of virus.

The rings were placed on the *Nicotiana glauca* stems about 1 inch below the point of union with the upper graft of *N. tabacum*. The rings were made as described for *N. tabacum* with the additions shown in table 6, where the results of this experiment are tabulated.

TABLE 6.—*Influence of rings on virus movement in Nicotiana glauca plants cut back to a height of 18 inches and grafted to curly-top Turkish tobacco at top and to healthy Turkish tobacco at base*

Number and position of rings	Plants inoculated	Effect on plants in which virus passed rings		Effect on plants in which virus did not pass rings	
		Plants affected	Average period between inoculation and appearance of symptoms below rings	Plants affected	Average period between inoculation and death of part above rings
	Number	*Number*	*Days*	*Number*	*Days*
Neither inner nor outer rings	10	10	23.6		
Outer ring only	15	15	23.8		
Inner ring only	15	15	22.7		
Outer ring 1 inch above inner, bud between	15	15	25.5		
Outer ring 1 inch below inner, bud between	15	15	22.5		
Outer ring 1 inch above inner, both in an internode	15	3	63.0	12	245
Outer ring 1 inch below inner, both in an internode	15	1	33.0	14	261
Outer and inner rings at same level in an internode	15	0		15	203
Outer and inner rings at same level, strip of bark in outer ring	10	10	19.5		
Outer and inner rings at same level, strip of phloem in inner ring	5	5	26.2		
Outer ring 1 inch above inner, both in internode, strip of bark in outer ring	6	6	19.6		
Outer [illegible] 1 inch below inner, both in internode, strip [illegible] bark in outer ring	5	5	20.0		

Plants having no rings required about the same average length of time for the appearance of symptoms on the lower graft as those in the groups having only an external or an internal ring. Two rings 1 inch apart seemed to have no influence in delaying the passage of virus in plants where the two rings were separated by a bud, regardless of their relative positions. The average length of time required for the appearance of symptoms was 25.5 days in plants where the outer ring was above the bud and 22.5 days in plants where the inner ring was above the bud. Whether this difference of 3 days is significant may be questioned. However, it is worthy of note that in the plants requiring the longer period for the appearance of symptoms on the lower graft the rings were so placed that materials moving downward in the phloem would be required to pass outward to the external phloem from the internal phloem through connection in the leaf traces.

Two rings, one external and the other internal, 1 inch apart, and both in an internode, prevented the passage of virus in 26 of 30 plants in the two series shown in table 6. In these two series, the 4 plants in which the virus passed the rings were of the same age and had been inoculated and ringed at the same time. At the time of inoculation they had relatively immature stems with thin woody cylinders. Serial sections of the internodes in which the two rings were located in all 4 plants revealed strands of newly formed tissue extending from the internal phloem at the top of the internal ring downward to the external phloem of the bark. Figure 7, *B*, shows a section of one of these strands.

In 15 plants having two rings at the same level in an internode, there was no instance in which the virus passed the rings. The average length of life of the diseased parts above the rings was 203 days. Figure 8, *A*, shows a plant of this series that retained virus above the ring for more than a year with no movement across the ring during this time.

The presence of a small strand of either internal or external phloem bridging rings which otherwise prevented virus passage, permitted the virus to pass with no measurable delay. The influence of a small strand of bark as compared with complete severing of phloem continuity is illustrated in figure 9, *A* and *B*. The influence of a bud between the internal and external rings is illustrated in figure 10, *A* and *B*.

RATE OF VIRUS MOVEMENT

A better understanding of the movement of virus in plants and of the factors influencing it would throw new light on some of the fundamental problems presented by plant viruses. The subject of virus movement in plants has received attention from several investigators. Sufficient evidence has been accumulated to indicate considerable variation in the behavior of different viruses. Whether this variation is due to the specific nature of the viruses or to the functioning of the plants in which they occur is a question of considerable interest and importance.

McCubbin and Smith (*18*) were among the first to present data on the rate of dispersion of viruses in plants. They measured the rate of movement of the virus of tomato mosaic by layering tomato plants, inoculating them at the distal end of one of the branches, and severing the stems between the rooted portions at different distances from the point of inoculation at known time intervals. By this means they were able to calculate a rate of movement of 1 to 2 inches per day or 1 to 2 mm per hour.

Severin (*22*) inoculated the distal ends of beet leaves with the curly-top virus by means of leaf hoppers and severed the inoculated leaves at measured distances from the point of inoculation after different time intervals. The most rapid movement measured by this means was 7 inches in 30 minutes, or a rate of 14 inches per hour.

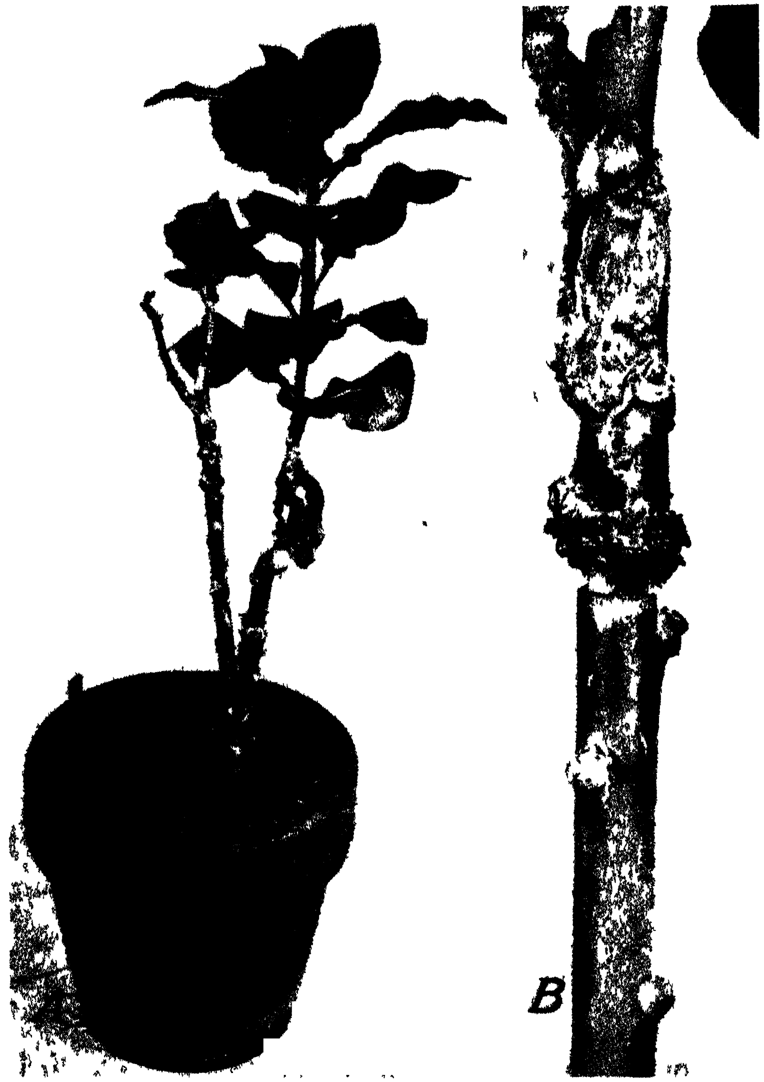

FIGURE 8.—*A*, *Nicotiana glauca* plant that had a diseased *N. tabacum* graft placed at the top and a healthy *N. tabacum* graft placed at the base and rings of internal and external phloem removed at the same level immediately below the top graft. After 420 days, virus was present in the part above the rings and absent in the parts below the rings. *B*, Ringed area, natural size. (Photographed 1 year after grafting.)

Storey (*27*), using methods similar to those of Severin, found that in 3 of 6 plants the virus of maize streak moved downward from the point of inoculation at the distal end of a maize leaf, a distance of 40 cm, in 2 hours.

Böning (*4*) found that the virus of tobacco mosaic moved 13 cm in 2 days in tobacco and 9 cm in 2 days in tomato.

Holmes (*15*), although not attempting an accurate measurement of maximum virus movement, has presented some very interesting studies on rate of invasion of tobacco plants by the mosaic virus.

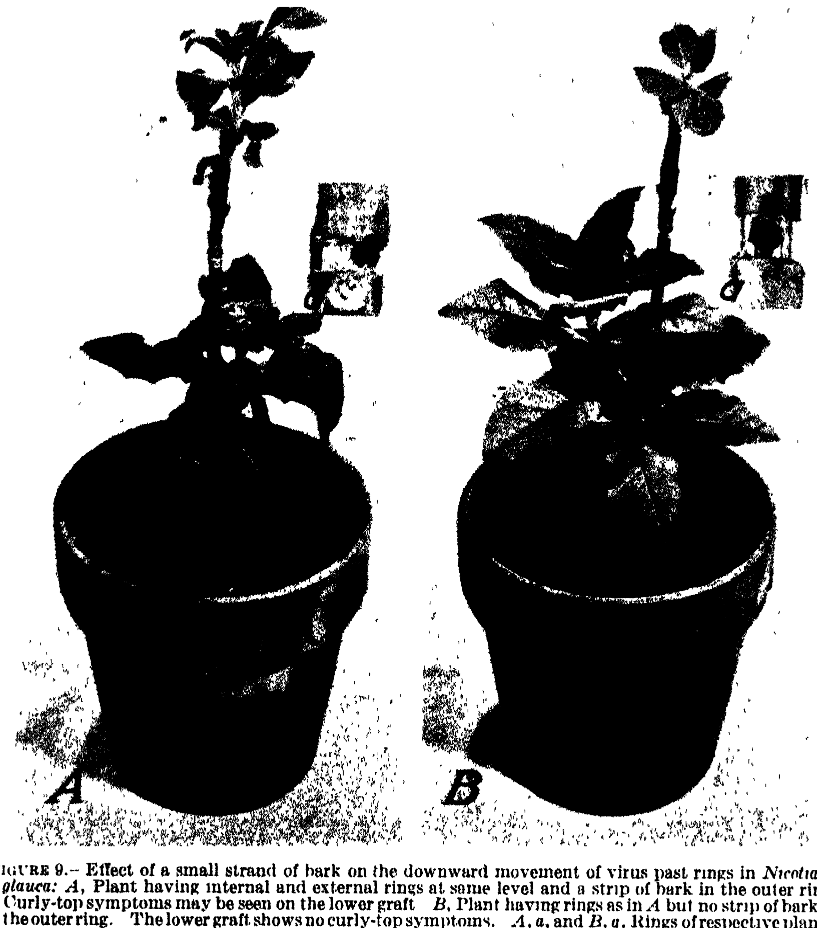

FIGURE 9.—Effect of a small strand of bark on the downward movement of virus past rings in *Nicotiana glauca*: *A*, Plant having internal and external rings at same level and a strip of bark in the outer ring Curly-top symptoms may be seen on the lower graft *B*, Plant having rings as in *A* but no strip of bark in the outer ring. The lower graft shows no curly-top symptoms. *A*, *a*, and *B*, *a*, Rings of respective plants, natural size. (Photographed 40 days after inoculation.)

He shows that the rate is at first very slow until the vascular bundle is reached. The virus moves more rapidly along the veins traversing the leaf blade and petiole. Apparently, the speed of movement is again accelerated when the virus passes into the stem from the inoculated leaf.

These results indicate a wide range in rate of movement among viruses in affected plants. This range extends from a rate of 1 to 2 inches per day in the tomato mosaic virus to a rate of 14 inches per hour in the virus of curly top. The factors determining these wide differences are of interest. Among the factors that may exert an influence at the time of testing are the specific nature of the tissue inoculated, the environmental conditions, the physiologic tone of the plant, and the species or variety of the plant. An effort has been made to extend the work done by Severin and to make further determinations on the rate of movement of the curly-top virus. Tobacco and sugar beet were used in these experiments.

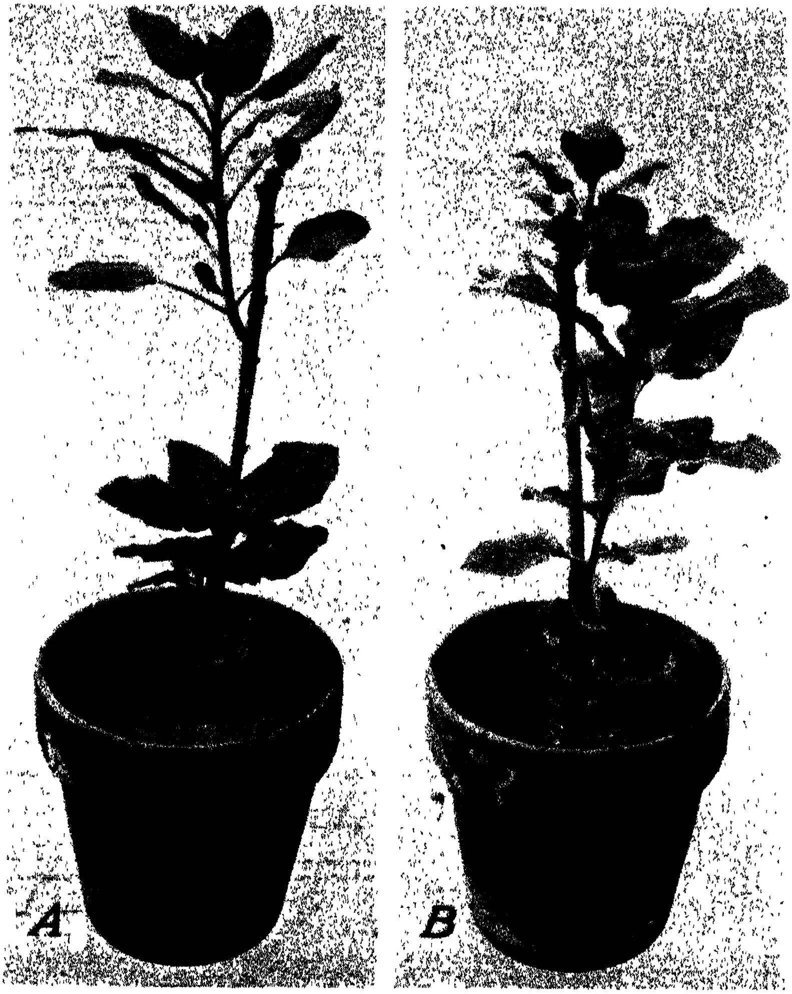

FIGURE 10.—Effect of a bud between internal and external rings on the passage of virus downward in a stem of *Nicotiana glauca*: *A*, Plant having external and internal rings and bud between. Note symptoms on the lower graft. *B*, Plant having internal and external rings in an internode. Note the absence of symptoms on the lower graft. (Photographed 40 days after inoculation.)

TOBACCO

Measurements were made of the rate of the downward movement of virus in the stems of the Turkish variety of *Nicotiana tabacum*. All the plants were more than 24 inches high at the time of their selection for this experiment, and some of them were showing the first indication of blossom buds. The youngest leaves of each plant were enclosed in a celluloid cage into which 50 to 100 viruliferous leaf hoppers were placed. The leaf hoppers were allowed to feed 5 hours.

The inoculated plants were incubated for different periods of time. At the end of the period allowed for virus movement they were cut off at a point 24 inches below the lowest point of inoculation, and all leaves were removed except the small ones on which the leaf hoppers fed. The stem was next cut into eight parts, each 3 inches long, and the segments placed in sand. In nearly all cases these cuttings rooted readily and produced a very satisfactory growth. The appearance of curly-top symptoms on a cutting indicated that the virus had reached that particular part of the stem in the period allowed for downward movement. The results of this experiment are shown in table 7. The virus did not move out of the inoculated 3 inches of the stem in any plant in 24 hours. In 48 hours the virus moved a distance of 24 inches in plant 10, or at a rate of one half inch per hour. The extent of stem invasion increased irregularly with the period allowed for virus movement up to 144 hours, when the virus had in all cases moved through the full 24 inches of stem and had reached the root of the plant. As calculated from these data, the maximum rate of virus movement is one half inch per hour (plant 10) for a 48-hour period.

Table 7 shows that there were decided differences among individual plants. For example, in plants 16 and 19 the virus had not moved out of the inoculated 3 inches in 96 hours, whereas in plant 17 it moved 24 inches in the same length of time. Perhaps the most interesting results were obtained from plant 20, in the 96-hour incubation period and from plants 23 and 24, in the 120-hour period. In plant 20 sections 1, 2, 3, 5, and 6 and the root portion were diseased and sections 4, 7, and 8 were healthy. In plant 23 sections 7 and 8 were diseased, whereas all the other sections were healthy, including the inoculated tip and the root below the 24-inch mark. In plant 24 sections 4, 5, and 7 were healthy and all the other sections were diseased. In all these plants, as usual in the experiment, the sections recorded as diseased showed symptoms on the first leaves from the buds and continued to show marked symptoms so long as they lived or until they were discarded. All the cuttings from these three plants were transferred to soil in 8-inch pots and the healthy-appearing ones were grown to flowering. The plants were then further tested for the presence of virus by grafting portions of the stems on healthy plants. In no case was evidence of the presence of virus obtained in plants grown from sections which had shown no symptoms. Symptoms were present at all times in all plants grown from other sections.

The significance of this erratic distribution of virus is not clear. In plants 20 and 24 it might well be that in its movement downward the virus failed to come into contact with tissue in segments 4, 7, and 8, and in segments 4, 5, and 7, respectively, in which it could become established and multiply. In plant 23, however, it is diffcult to account for the absence of virus in the inoculated portion and its presence in two segments farther down the stem.

TABLE 7.—*Rate of downward movement of curly-top virus in stems of Turkish tobacco (Nicotiana tabacum)*

Plant no.	Period between inoculating and making cuttings	Infection [a] of indicated 3-inch cutting [b] and root								
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Root
	Hours									
1	24	+	−	−	−	−	−	−	−	−
2	24	+	−	−	−	−	−	−	−	−
3	24	+	−	−	−	−	−	−	−	−
4	24	+	−	−	−	−	−	−	−	−
5	24	+	−	−	−	−	−	−	−	−
6	48	+	−	−	−	−	−	−	−	−
7	48	+	−	−	−	−	−	−	−	−
8	48	+	+	+	−	−	−	−	−	−
9	48	+	+	−	−	−	−	−	−	−
10	48	+	+	+	+	+	+	+	+	+
11	72	+	+	−	−	−	−	−	−	−
12	72	+	−	−	−	−	−	−	−	−
13	72	+	−	−	−	−	−	−	−	−
14	72	+	+	+	−	−	−	−	−	+
15	72	+	+	+	+	+	+	+	+	+
16	96	+	−	−	−	−	−	−	−	−
17	96	+	+	+	+	+	+	+	+	+
18	96	+	+	+	+	+	+	+	+	+
19	96	+	−	−	−	−	−	−	−	−
20	96	+	+	+	−	+	+	−	−	+
21	120	+	−	−	−	−	−	−	−	−
22	120	+	+	+	+	+	+	+	−	−
23	120	−	−	−	−	−	−	+	+	−
24	120	+	+	+	−	−	+	−	+	+
25	120	+	+	+	+	+	+	+	+	+
26	144	+	+	+	+	+	+	+	+	+
27	144	+	+	+	+	+	+	+	+	+
28	144	+	+	+	+	+	+	+	+	+
29	144	+	+	+	+	+	+	+	+	+
30	144	+	+	+	+	+	+	+	+	+

[a] Plus and minus signs indicate positive and negative results, respectively, obtained from planting various 3-inch sections of the inoculated stem. Where a cutting showed infection it was considered to indicate that the virus had reached that section of the stem in its downward movement.

[b] Counting downward from inoculated top.

SUGAR BEET

SEEDLING BEETS

Experiments with seedling beets were planned with a view to studying the rate of movement of virus in cotyledons under different conditions of temperature. Seedling plants were used that had the beginning of first true leaves and cotyledons more than 1 inch long. Leaf hoppers were caged singly on the tip of one cotyledon of each plant. The time at which each leaf hopper started to feed was noted and the feeding period was terminated after the desired interval by removing the leaf hopper. The plants were grown in 6-inch pots, 4 plants per pot. The pots were numbered consecutively. In all even-numbered pots the cotyledons on which the leaf hoppers fed were severed 1 inch from the point of feeding after the desired period allowed for virus movement. The odd-numbered pots were kept as controls and received the same treatment as the even-numbered pots, except that the cotyledons on which the leaf hoppers fed were not removed. The feeding periods of the leaf hoppers were 2, 3, and 5 minutes; the periods allowed for virus movement were 2, 3, 5, 10, and 15 minutes; and the air temperatures at which the various tests were made were roughly 60°, 85°, 110°, and 135° F. The results of these experiments are shown in table 8.

TABLE 8.—*Rate of movement of virus in cotyledons of young sugar-beet plants*

Temperature (°F.)	Leaf-hopper feeding period	Period allowed for virus to move 1 inch	Cotyledon removed			Cotyledon not removed		
			Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	Minutes	*Minutes*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
60	2	2	80	0	.0	79	0	.0
	3	3	80	0	.0	80	0	.0
	5	5	80	1	1.2	80	9	11.2
	5	10	66	5	7.5	71	11	15.4
	5	15	77	5	6.4	82	6	7.3
85	2	2	176	0	0	183	9	4.9
	3	3	81	15	18.5	79	24	30.3
	5	5	70	8	11.4	76	19	25.0
	5	10	74	14	18.9	74	17	22.9
	5	15	73	13	17.8	75	21	28.0
	5	60	80	11	13.7	80	15	18.7
110	2	2	80	2	2.5	80	8	10.0
	3	3	78	6	7.7	80	11	13.7
	5	5	82	32	39.0	78	31	39.7
	5	10	68	18	26.4	64	12	18.7
	5	15	80	26	32.5	80	25	31.2
135	2	2	80	1	1.2	80	6	7.5
	5	5	40	8	20.0	40	10	25.0

Infection did not occur in any plant after the 2- and 3-minute feeding periods in the 60° series nor in any plant after the 2-minute feeding in the 85° series. Infection occurred in test plants and controls after all the other feeding periods and exposures in all series. As calculated from these data, the rates of movement were as follows: At 60°, 12 inches per hour; at 85°, 20 inches per hour; at 110°, 30 inches per hour; and at 135°, 30 inches per hour. However, considerable caution should be exercised in drawing conclusions from these calculations regarding the effect of temperature on the rate of virus movement. Although the calculated rate was lowest at 60°, the percentage of infection was also low. The low percentage of infection indicates a lower inoculative efficiency on the part of the vector, which in turn may mean that the minimum time required for infection to occur may be longer than at higher temperatures, thus correspondingly reducing the available period for virus movement. However, the results at 110° seem to furnish some support for the assumption that the virus moves more rapidly at this temperature than at 60° or at 85°, since in the 5-, 10-, and 15-minute exposures, as measured by the controls, the removal of the cotyledons on which the leaf hoppers fed seemed to have little influence on the percentage of plants which later developed disease; whereas at 60° and 85° the percentage of infection was considerably reduced by removing the cotyledons after exposure.

BEET PLANTS HAVING SEVERAL TRUE LEAVES

In seedling plants the determination of very rapid rates of virus movement is limited by the minimum duration of the required infection period and by the length of the cotyledons. In order to obtain a relation between time of infection and extent of movement which would permit the detection of rates of movement more rapid than 30 inches per hour, plants having leaves 3 to 10 inches long were used in a second experiment. Ten leaf hoppers were allowed to feed 6 minutes at the distal end of a leaf on each plant at a temperature of 85° to 100° F. In the odd-numbered pots the inoculated leaves were severed 1, 2, 3, etc., up to 10 inches from the point of inoculation 6 minutes after feeding started. The even-numbered pots were retained as controls. The experiment was run in three series (table 9). The results within a series are considered comparable, but one series is not strictly comparable with any other, because of differences in date of inoculation and in age of plants used.

The results of this experiment show a maximum downward movement of 6 inches in 6 minutes, or a rate of 60 inches per hour. An analysis of results indicates that even this rapid rate of movement does not represent the maximum rate attainable under the most favorable conditions for movement. Series 1, 2, and 3 of table 9 show considerably less infection in plants from which the inoculated leaf was removed than in the controls. This, however, did not hold in some of the subsequent tests made on very rapidly growing plants.

Experiments 1 and 2 of table 9 show the results of two additional tests on plants growing at different rates at the time of inoculation. The plants used in experiment 1 of this table were thrifty but were not growing at an excessive rate; those used in experiment 2 were in rich soil and were growing very rapidly. In experiment 1, infection in the test plants was considerably less than in the controls. Moreover, infection decreased as the distance of required virus movement increased. The results of this experiment, if standing alone, would indicate (1) that there was a rather uniform decrease in infection as the length of leaf removed was increased and (2) that the virus would not move more than 4 inches in 6 minutes.

TABLE 9.—*Virus movement in leaves of young sugar-beet plants*

Series or experiment	Length of leaf removed	Inoculated leaf not removed			Inoculated leaf removed			Calculated rate of virus movement per hour
		Plants inoculated	Plants infected		Plants inoculated	Plants infected		
	Inches	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Inches*
Series 1	1	120	56	46.6	120	27	22.5	10
	2	120	64	53.3	120	33	27.5	20
	3	120	50	41.6	120	20	16.6	30
Series 2	4	140	38	27.1	140	15	10.7	40
	5	140	46	32.8	140	15	10.7	50
	6	140	47	33.5	140	17	12.1	60
Series 3	7	40	10	25.0	40	0	0	----------
	8	40	11	27.5	40	0	0	----------
	9	40	10	25.0	40	0	0	----------
	10	40	10	25.0	40	0	0	----------
Experiment 1	1	20	17	85	20	8	40	10
	2	20	13	65	20	6	30	20
	3	20	15	75	20	3	15	30
	4	20	11	55	20	1	5	40
	5	20	7	35	20	0	0	----------
	6	20	6	30	20	0	0	----------
Experiment 2	1	20	13	65	20	8	40	10
	2	20	14	70	20	10	50	20
	3	20	10	50	20	10	50	30
	4	20	13	65	20	11	55	40
	5	20	12	60	20	11	55	50
	6	20	12	60	20	9	45	60

In rapidly growing plants, results were quite different. The removal of the inoculated leaf after 6 minutes did not appreciably decrease the percentage of infection as measured by the controls. This was true regardless of whether 1, 2, 3, 4, 5, or 6 inches of leaf was removed, and the chances, therefore, that the virus would move 6 inches in 6 minutes were about the same as that it would move 1 inch in 6 minutes. It seems probable that under the conditions of this experiment virus could have been shown to move more than 6 inches in 6 minutes had longer leaves been available.

DISCUSSION

The results obtained in the various experiments outlined in this paper are interpreted as indicating a very intimate relationship between the curly-top virus and the phloem tissue of affected plants. Virus is introduced into the phloem by its insect vector. It moves downward from the point of inoculation toward the root system at a very rapid rate. Results of leaf-hopper feeding experiments indicate that the virus concentration in exudate, believed to be derived largely from the phloem, is high as compared with that in expressed juice from the entire beet. Virus apparently does not pass from the xylem into the phloem or any other tissue in sufficient quantities to cause systemic infection. After it is established in the phloem the evidence indicates that it passes into adjacent parenchyma tissue only in very limited amounts in the beet, and experiments have failed to demonstrate that it ever occurs in the parenchyma of two species of tobacco. The evidence supporting these conclusions may be worthy of further study.

It is considered probable that the close association of the virus with the phloem tissue may have a bearing on the difficulty encountered in obtaining infection by mechanical inoculation. The ordinary methods of mechanical inoculation undoubtedly can be depended upon to introduce virus into parenchymatous tissue of various kinds, since infection is induced readily with other viruses by these methods. Artificial-inoculation experiments indicate that introduction of virus into such parenchyma cells as the leaf hairs of beet, tobacco, squash, or bean, never produces infection. Since the virus does not set up a systemic infection after its introduction into parenchymatous cells, only two other possible courses are open to it. It must either remain in these cells and fail to set up a systemic infection or it must be inactivated by substances resulting from cell injury or by normal constituents of parenchymatous cells.

In the light of the information now available, it seems probable that if artificial infection is to be obtained in any appreciable percentage of the plants inoculated the virus must be introduced directly into the phloem. Clearly the introduction of virus into phloem by mechanical means is attended with difficulty. Needles that are available for introducing virus into plants are large enough to crush many cells, and it is probable that the phloem is so badly injured in the process of inoculation that the virus does not often become established. Even if needles sufficiently fine to penetrate the phloem without causing excessive injury were available, they would probably still fall far short of the insect vector in effectiveness. Such a needle would necessarily carry the virus on the surface, where it would be exposed to the action of contents of parenchyma cells and to depletion in the passage of the needle through tissue exterior to the bundle. Once the needle penetrated the phloem, a portion of the virus might be liberated. If the needle were withdrawn, the phloem content, being under a positive pressure, would pass quickly into the cavity that was left, probably carrying a part or all of the virus deposited in the phloem back into the region of parenchyma.

The best available method of artificial inoculation seems crude when compared with the refinements introduced by the insect vector. The leaf hopper's stylets are extremely slender and seem to find the phloem with remarkable accuracy. As the stylets pass through the parenchymatous regions exterior to the phloem, a protective sheath is laid down which may effectually exclude contents of surrounding cells. After the phloem is punctured the insect remains in a feeding position for appreciable periods and may leave considerable deposits of sheath material in the vascular area. As the stylets are removed the puncture is probably completely plugged by sheath material.

Since virus is introduced into the phloem directly by the leaf hopper, its movement to certain parts of the beet plant probably starts immediately and is very rapid. Probably the phloem of the growing point is invaded in a few hours and the entire phloem network of the plant is invaded in a few days. Evidence indicates that under some conditions the virus is closely restricted to the phloem, under other conditions it may escape into the intercellular spaces of the parenchyma. This is shown by the occurrence of drops of exudate having a high virus content on the petioles of badly diseased beets. Esau (*13*) has shown that this exudate moves from the vicinity of the phloem to the exterior of the petiole through the intercellular spaces. The causes of such movements are not clearly understood.

Crafts (*9*) states that the phloem is normally under a positive pressure and suggests that the cambium on one side and the starch sheath on the other limit lateral movement of phloem content. It is possible that phloem necrosis and the presence of regenerative tissue may interfere with the movement of solutes and increase the pressure in the phloem of diseased beets. This increased pressure may be sufficient to force phloem content through the limiting layers into the intercellular spaces of the parenchyma and to the surface of the petiole, or, as Esau has suggested, the presence of the virus may render the limiting layers more permeable to phloem content. If the cells of the limiting layer are rendered more permeable it seems probable that there might be a seepage of virus from phloem tissue throughout the plant. If the virus is not extruded through breaks in the limiting layer it may pass through cells that are rendered more permeable. Whether the virus is able to pass from the intercellular spaces into the cells of the parenchyma seems doubtful. The failure to obtain infection by introducing virus into parenchyma cells and the rapid inactivation of virus in expressed juice point to parenchyma as being a very poor medium for virus. The failure of leaf hoppers to acquire virus from any type of parenchyma except that immediately below the crown and in one instance from parenchyma of the petiole indicates that the virus content of parenchyma is very low. The relatively small amount of virus obtained by leaf hoppers from expressed juice as compared to that obtained from phloem exudate indicates that the juice is low in virus content or that the leaf hoppers are much less efficient in acquiring virus from expressed juice than from phloem exudate.

The weight of evidence seems to indicate that the virus content of parenchyma of beet is very low. The virus present in parenchyma may occur largely or exclusively in the intercellular spaces.

It seems probable that the virus may be even more closely restricted to the phloem in the two species of tobacco tested than in sugar beet. This is indicated by results of experiments in which the virus was held in inoculated parts in plants in which its further dispersal was dependent upon its ability to pass through a small amount of parenchymatous tissue. In most instances this parenchymatous bridge was composed of only a few cells, as in plants in which two rings, an internal and an external, 1 inch apart, were placed in an internode. In plants having the internal ring above the external the virus would move down the stem in the external phloem 1 inch past the internal ring, thus affording an area equal to the linear distance between the rings multiplied by the circumference of the internal ring for inward movement of virus through the wood or medullary rays to the internal phloem in which it could pass on down the stem. In plants having the positions of the two rings reversed the required movement would be from the internal to the external phloem through the wood or medullary rays. When it is considered that in these experiments active virus was held in the parts above the rings, in some instances for more than a year, with no leakage whatever of virus across the rings, the effectiveness of this barrier is apparent. To explain this phenomenon it is necessary again to assume that the virus does not pass out of the phloem or that it is very quickly inactivated by contact with normal cells of other tissues.

The close association of the curly-top virus with phloem tissue may have an important bearing on the failure of beet seeds to transmit disease. Artschwager (*1*) has shown that in the development of the beet seed there is no direct vascular connection between the mother plant and the young sporophyte. Therefore, materials that enter the embryo must do so by diffusing through a layer of meristematic or parenchymatous tissue. Results of experiments showing a lack of movement of virus through meristem and parenchyma indicate that such a layer would offer a formidable barrier to passage of the virus into the embryo, and might easily account for the absence of seed transmission of this disease.

Just how far it is safe to venture in applying this principle to other virus diseases is difficult to say. It would seem to go far toward explaining lack of seed transmission in all diseases in which the virus is restricted to the phloem. It may be important for other virus diseases. Nelson (*20*) has suggested such an explanation for the erratic transmission of bean mosaic. The virus of this disease is not known to be restricted to the phloem. It occurs in only a part of the seeds from diseased plants. In studying the development of the bean seed, Nelson found no direct vascular connection between the mother plant and the embryo. He suggests that the virus may be able to pass into the embryo through the nonvascular-containing layer only when this layer is in a meristematic stage of development. The failure of certain seeds to carry virus is accounted for by assuming an incomplete distribution of the virus in the phloem. Even with a complete virus invasion of the phloem, which may seem more likely in plants grown from diseased seeds, it would seem reasonable to suppose that the passage of virus through meristem or parenchyma of this type may be a hazardous one and successfully accomplished only under certain conditions. It is possible that the meristematic or parenchymatous layer of tissue separating the mother plant from the embryo may offer a structural or chemical barrier to virus passage even with such viruses as that of tobacco mosaic, which is known to occur in certain types of parenchymatous tissue.

The rate of movement of virus in sugar-beet leaves is so rapid as to call for a consideration of the mechanics of this phenomenon. Since it seems fairly obvious from data already presented that this movement occurs in the phloem, the possibilities inherent in the solution of this problem are all the more interesting. Under conditions of the greatest measured rate of virus movement in sugar beet (6 inches in 6 minutes) it seems safe to conclude that there is no appreciable multiplication of virus in the plant in so short a time, and it would not seem that multiplication would be an appreciable accelerating force in movement. That such movement may result from any autonomous effort on the part of the virus particles seems out of the question, since this rate is several times greater than the most rapid movement of the swiftest moving micro-organisms known. Simple diffusion is infinitely slower than the measured rate of virus movement. Diffusion accelerated by protoplasmic streaming in the sieve tubes could account for no rate approaching maximum movement.

Certainly none of the foregoing theories will account for this rate of movement, regardless of whether virus is considered to be a living organized entity or a chemical compound. In spite of these facts it is now pretty definitely known to physiologists that certain substances move in phloem at a very rapid rate of speed. Mason and Maskell (*19*) calculate a rate of movement of sugars in the cotton plant equal to the rate of diffusion of molecules of that size in air. Crafts (*10*), using figures derived from growth increase in pumpkin and cucumber over a stated period, estimates an average linear rate of movement of 0.292 and 0.235 cm per minute, respectively, of materials through the stem into the fruits. By cutting stems of cucumber and measuring phloem exudate, he found that a calculated rate of movement of phloem content varying between 3.64 and 8.62 cm per minute could be induced. Crafts (*9*), in discussing food translocation, suggests that a mass movement of elaborated food materials takes place in the phloem. This mass movement he conceives as being dependent on the creation of a pressure gradient due normally to the increased osmotic pressure in the more active photosynthetic areas of the plant. This high osmotic pressure accelerates the intake of water, which in turn increases the hydrostatic pressure in the phloem and causes phloem content to move to parts where the pressure is lower. In such a system each compound would not move independently of other compounds as in simple diffusion, but would move at a rate approximating that of the mass as a whole, assuming no selective interference in the path of movement.

Accepting this hypothesis of food translocation, we have available a hypothesis of virus movement which seems to satisfy all the known conditions of such movement. In a virus movement of 6 inches in 6 minutes it seems unlikely, as stated previously, that virus increase would be an important factor under any conditions. Therefore virus concentration during the first few minutes after introduction is probably very low, and the virus itself could not function to increase appreciably the osmotic concentration of materials at the point of introduction. However, if the hydrostatic pressure of the phloem at the point of virus introduction were already high because of an abundance of photosynthates, and these photosynthates were being transported at a very rapid rate, the introduced virus particles would be carried along at a rate corresponding to the rate of food flow, provided of course that mechanical interference were the same for each. Under such conditions the virus would move at the same rate and in the same direction as elaborated foods in the phloem and would in fact be an indicator of the rate and direction of food translocation.

SUMMARY AND CONCLUSIONS

Sugar-beet plants were induced by various treatments to take up juice of beets affected with curly top through the water-conducting channels, but since none of the plants so treated became infected, it is evident that the curly-top virus does not pass from the tracheae into cells or tissues where it can become established and initiate pathologic symptoms.

Several other methods of inoculation were tried with inocula prepared in different ways, but an appreciable percentage of infection was obtained only when the phloem exudate of a curly-top beet was used as the inoculum.

The vector of the curly-top virus, *Eutettix tenellus*, feeds on the leaf veins, and its mouth parts usually penetrate the phloem region. As the mouth parts are inserted the insect lays down a sheath of apparently gelatinous material which completely incases the stylets. This sheath may seal off all cells penetrated that are external to the phloem, and thereby protect the virus as it is passed into or drawn out of the phloem by the leaf hopper.

The higher mortality rate in groups of leaf hoppers on parenchyma tissue, as compared with mortality in groups on tissue containing vascular elements, indicates that parenchymatous tissue does not serve as a favorable source of food. However, leaf hoppers having access to parenchyma lived longer than those given neither food nor water, indicating that they extracted a certain amount of material from parenchyma. Leaf hoppers given access to parenchyma tissue of petioles and crowns and to pith and immature seeds, all from diseased beets, and then caged in groups of 5 on healthy beet seedlings, infected only 10 of 428 plants. An equal number of insects from tissue containing vascular elements infected 210 of 428 plants. These results supplement other evidence supporting the view that virus is concentrated in the phloem and is present only in relatively small amounts in the parenchyma. Since in sugar beets affected with curly top, phloem content escapes into the intercellular spaces of parenchyma tissue surrounding the vascular bundles, it is suspected that the virus recovered from the parenchyma may have been derived from escaped phloem content.

The exudate occurring on the petioles and blades of beets affected by curly top and that from the cut surface of affected beets has a high virus content. Evidence indicates that the exudate that occurs naturally on the petioles and blades is derived largely from the phloem and that the exudate from the cut surface of the beet is derived from the phloem except as it may be contaminated by an undetermined amount of material from the xylem and from injured cells mainly parenchymatous in nature.

Healthy sugar-beet and tobacco plants were infected by grafting diseased plants on them. Beets were not infected when union of the grafted plants did not result. In the grafted tobacco plants a definite union consisting of meristematic tissue was found after 3 days. In unions 7, 8, and 9 days old, tracheal elements were apparently mature and strands of elongated cells paralleling the tracheae probably included functional phloem. No infection resulted until the seventh day. Infection increased from 27 percent on the seventh day to 100 percent on the twelfth day. In view of the fact that the virus does not gain effective entrance through the tracheae and did not in these experiments pass through meristematic or parenchymatous tissue, it must have passed the graft union through the phloem elements.

Ringing experiments with *Nicotiana tabacum* and *N. glauca* showed that the virus passes all rings bridged by an uninterrupted path of phloem, internal or external or internal and external combined. The virus failed to move past the rings when the internal and external phloem were removed at the same level. Interruption of phloem continuity by rings placed at different levels in an internode prevented the passage of virus in 20 of 27 *N. tabacum* plants and in 26 of 30 *N. glauca* plants. Serial sections of the ringed area in all the plants in which the virus passed the rings showed in each case one or more areas of regenerated tissue connecting the internal and external phloem through the woody cylinder. In view of these findings it seems probable that virus does not move longitudinally or radially through any of the normal elements of the woody cylinder and that dispersal in these two species of tobacco is dependent on the presence of continuous phloem elements.

The movement of the curly-top virus in tobacco is relatively slow as compared to the movement in sugar beet. The fastest movement observed in tobacco was downward from the point of inoculation at the top of the plant to a point 24 inches below in 48 hours; a rate of movement of one half inch per hour.

In sugar beet the virus moves at a much more rapid rate. At air temperatures of approximately 85°, 110°, and 135° F. the virus moved outward in cotyledons from the point of inoculation a distance of 1 inch in 2 minutes. In larger beets the virus moved downward from the point of inoculation at the distal end of a leaf to a point 6 inches below in 6 minutes, a rate of movement of 60 inches per hour. These rapid movements of virus evidently occur in the phloem and it is suggested that they indicate a rapid translocation of plant materials. For these reasons virus may prove useful as an indicator in studies on the movement of elaborated foods.

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CHEMICAL COMPOSITION AND YIELD OF THE ALASKA PEA AS INFLUENCED BY CERTAIN FERTILIZERS AND BY THE STAGE OF DEVELOPMENT[1]

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INTRODUCTION

It is believed by many canners and growers of peas for the cannery that if potash in appreciable amounts is present in the fertilizer applied to a pea crop the peas will mature earlier and be harder, more starchy, and of lower quality at a given stage of development than if no potash or only a small amount is used. Boswell (*3*, *4*)[2] conducted fertilizer experiments in three different parts of Maryland over a period of 3 years and used, among other materials, muriate of potash alone and in combination with nitrogen and phosphorus. All treatments were duplicated each year. Although he made no chemical analyses of the peas, field observations led him to conclude (*4*, *p. 352*) that—

> Muriate of potash, alone or in combination with nitrogen and phosphorus, has given no consistent results with respect to yield, rate of maturity, or apparent condition of the crop at harvest. The opinion that potash hastens maturity and lowers the quality of the crop has not been borne out in this work.

Later Sayre and his associates (*23*) made exhaustive chemical and physical examinations of peas grown under a great variety of fertilizer treatments in the field and in water cultures. They devoted special attention to the effect of potash upon rate of maturity and quality of peas. In their conclusions it is stated: "In spite of this wide variety of conditions, only minor differences in quality of peas *of the same stage of growth* have been obtained." They further conclude: "Thus far, the belief that potash has a harmful effect upon the quality of the crop appears to be ill-founded."

Earlier work of one of the present writers (*4*) indicated the desirability of determining by more accurate methods than had previously been used the effect of potash and other fertilizer constituents upon the development of the pea as indicated by its chemical composition. Nitrogen fertilizers appeared to be of special interest, since earlier observations had suggested that a generous use of available nitrogen tends to delay maturity and to improve the quality of the pea. Accordingly, studies were made to determine the effect of certain fertilizers and of the stage of development on the composition and yield of peas.

GROWING AND HANDLING EXPERIMENTAL MATERIAL

FIELD-PLOT METHODS

The Alaska variety of pea (*Pisum sativum* L.) was used in these studies. Work was carried on at the Arlington Experiment Farm,

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[2] Reference is made by number (italic) to Literature Cited, p. 735.

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Rosslyn, Va., in 1930 and 1931. All treatments were in duplicate, and each consisted of an application of a single fertilizer constituent. The treatments and rates of application per acre were as follows:

N.—Nitrate of soda 400 pounds and sulphate of ammonia 280 pounds (about 116 pounds of nitrogen).
P.—Superphosphate 1,000 pounds (about 160 pounds of phosphoric acid).
K.—Muriate of potash 300 pounds (about 144 pounds of potash).
C.—Check; no treatment.

The quantities of fertilizers used per acre were in excess of those employed in growing peas for the cannery, the object being to produce measurable differences in the composition of the products. In order to overcome as far as possible any definite gradient in soil conditions that might exist, the eight plots were arranged as follows:

Potash	Check
Phosphorus	Nitrogen
Check	Phosphorus
Nitrogen	Potash

The fertilizing materials were applied by hand after the soil had been plowed and disked once. They were then worked in by further disking and harrowing, care being taken to avoid dragging soil from one plot to another. The peas were sown with a garden drill at a depth of 1 to 1½ inches.

In 1930 the plots were located on a moderately fertile area of artificial land which had been dredged from the Potomac River and which was apparently of a silt-loam character. Each plot was 53 by 33 feet. The rows were 14 inches apart, sown at the rate of approximately 4 bushels of seed per acre. A border of 1 row along the sides and a width of 1 foot at the ends of each plot was left unharvested. In 1931 the same plots and treatments were used as in 1930. One tier of 4 plots was sown in rows 7 inches apart, at the rate of 4 bushels of seed per acre; the other tier was sown in 14-inch rows, at the rate of 2 bushels per acre. In 1930 the fertilizers were applied and seed sown on March 18, and the peas were harvested on May 28 and on June 2. In 1931 the plots were fertilized and sown on April 10 and the peas harvested on June 6 and June 10.

HARVESTING, GRADING, AND DRYING

In both 1930 and 1931 a power-driven pea huller, a hand-driven grader, and a specially constructed drier not only permitted the use of large and adequate samples but also very greatly reduced the time from harvest to complete dryness of the material.

The drier consisted of an asbestos-board cabinet 6 feet high, 4½ feet wide, and 2½ feet deep, inside measurement, with the entire front made of double doors which opened at the center. The outside was covered with a one half inch layer of builders' insulating material. Ten shallow trays 4 feet long and 2½ feet wide rested upon horizontal iron-rod supports. The bottoms of the trays were of 8-mesh hardware cloth. Each tray was supported by three three-eighth inch rods which prevented the sagging of the center of the large screen-wire bottom. There was a 3-inch "head space" between trays. The opposite ends of alternate trays were placed against the side walls when the drier was in operation, thus forcing the hot air to travel over each tray that carried material to be dried.

Immediately to one side of the cabinet and connected with it by an opening near the base were two low-form, five-column, common steam radiators each of 50 square feet radiation. Over these radiators, which were enclosed in an asbestos-board housing, a strong current of air was forced by a centrifugal blower of a capacity of 1,500 cubic feet per minute driven by a three-horsepower electric motor. With a steam pressure of 15 pounds per square inch in the radiators, a temperature of 60° to 65° C. could be maintained in the cabinet during the drying of a considerable mass of plant material. In the brief period of 3 to 4 hours 25 to 30 pounds of peas were dried to such a degree that they rattled when handled. The material to be dried had to be spread very thinly and uniformly on the trays and moved carefully about from time to time to obtain satisfactory results.

At each harvest date the peas from one half of each plot were gathered; all pods were removed from all plants scheduled for harvest on that date. The duplicate or second series of plots was harvested in reverse order to the first, since all plots could not be harvested simultaneously. In most instances the samples of shelled peas were placed in the drier within 3 to 4 hours after the pods were harvested.

FIELD RESULTS

YIELD

The yield data are of secondary importance, since the treatments were made for a specific purpose other than for increasing yields and were of a character that ordinarily would not be recommended in field practice. However, the yield data, presented in table 1, indicate the growth relationships due to the various treatments and the general level of nutritional conditions under which the studies were made. Individual plots of the duplicate treatments are designated A and B.

Even though small differences appear between the total yields of fresh shelled peas from the various treatments in 1930 and 1931, they are of no significance, for the differences between duplicates are in general greater than between treatments. The absence of material differences in yield is not what one might expect but possibly may be accounted for in part by (1) the high fertility of the soil to which treatments were applied, and (2) by injury from excessive amounts of readily soluble nutrient salts. The latter suggestion carries little weight because no definitely injurious effects were noted except in the nitrogen-treated plots of 1930. Furthermore, in market-garden areas peas are heavily fertilized without injury. A late frost in 1930 injured the nitrogen-treated plots rather severely, but the others were not appreciably harmed. Further evidence of the lack of fertilizer injury was afforded by yields of similar plots in 1931. Germination of the pea is known to be adversely affected by excessive concentrations of fertilizer salts, but no damage was noted in any of these plots.

TABLE 1.—*Yield per acre of shelled peas (fresh weight) as affected by potash, phosphoric acid, and nitrogen in 1930 and 1931*

1930 CROP

Harvest and grade no.	Plot	Yield from treatment indicated			
		Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:		*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	A	98	158	167	51
	B	118	112	179	135
2	A	182	146	218	79
	B	236	292	250	109
3	A	149	58	195	103
	B	136	374	213	73
4	A	39		48	19
	B	30	68	41	31
5	A			12	24
	B				
All grades	A	468	362	640	276
	B	520	846	683	348
Average, A and B		494	604	662	312
Harvest of June 2					
1	A	85	75	86	71
	B	75	99	74	37
2	A	185	168	144	80
	B	140	212	141	120
3	A	313	234	601	391
	B	434	352	573	155
4	A	401	154	321	196
	B	185	328	567	173
5	A	145	80	68	
	B	112	136		
All grades	A	1,129	711	1,220	738
	B	946	1,127	1,355	485
Average, A and B		1,038	919	1,288	612

1931 CROP

Harvest and grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 6:					
1	A	104	164	215	122
	B	128	176	202	104
2	A	261	287	352	289
	B	303	512	444	303
3	A	234	157	192	265
	B	257	396	289	208
4	A	63	22	35	99
	B	40	84	42	36
All grades	A	662	630	794	775
	B	728	1,168	977	651
Average, A and B		695	899	886	713
Harvest of June 10:					
1	A	127	137	198	105
	B	320	212	351	260
2	A	402	382	414	316
	B	761	646	928	626
3	A	678	558	934	592
	B	943	1,166	994	882
4	A	542	462	724	580
	B	388	700	352	358
5	A	17	19	57	28
	B		22	8	12
All grades	A	1,766	1,558	2,327	1,621
	B	2,407	2,746	2,638	2,138
Average, A and B		2,087	2,152	2,480	1,880

In 1930 phosphoric acid produced a definite increase in yield. In 1931 the same plot with the same phosphorus treatment also showed an increase of appreciable magnitude (table 1).

It should be borne in mind that these increases in yield were obtained on a soil dredged from the river. In past years it had received moderate applications of manure and green manure and may have been relatively low in phosphorus. Other field experiments in nearby Maryland have not shown phosphorus to be markedly effective in increasing the yield of peas. As previously explained, the low yield of the nitrogen plot in 1930 is believed to have been due to frost injury while the peas were in an early stage of growth. In 1931 nitrogen and potash applications resulted in no significant differences in yield.

MATURITY

In this investigation the possible effect of nutrients upon rate of development and properties of the peas is of more interest than the effect upon yield.

In this work the rate of development or stage of maturity has been judged on the basis of distribution of sizes of peas in the pods. This is the basis used by canners' field men in determining when a field has attained the proper stage for harvesting. In order to reduce the expression of maturity to a single numerical value, the percentage of the yield of a plot constituted by each grade was multiplied by an arbitrary weighting, for convenience, equal to the standard grade number. The sum of these products of a single harvest is designated as a "maturity index" (*4*). Sayre and his associates (*23*) have suggested, since the present work was begun, that as an index to quality this maturity index is less reliable than their "quality index." The latter is calculated by summing the products obtained in multiplying the percentage of yield constituting each grade by the numerical value of the crushing test for that grade. However, since stage of development is commonly judged by distribution of sizes, and since this work was designed to study the pea as generally handled in field practice, the above-described maturity index is believed to be better adapted to the present investigation.

Table 2 shows the distribution of sizes of shelled peas of each harvest from each plot as well as the maturity indices for each plot and for the total yield of duplicate plots of each treatment. No appreciable consistent differences in maturity resulted from the treatments. The same was true when adjacent different treatments were compared in the field. In one comparison the difference was in favor of one plot, and, more often than not, the relationship was reversed in the second comparison. When the indices for the total plot area of each treatment are compared, they are found to be quite similar for any one harvest date.

There was no evident difference in the time of blossoming of the plants in any of the several plots. Thus, earlier observations in the field, and the reports of other investigators (*4*, *23*), appear to be confirmed under additional and quite different conditions as reported in this paper.

TABLE 2.—*Influence of potash, phosphoric acid, and nitrogen upon stage of maturity of peas at harvest in 1930 and 1931*

1930 CROP

Harvest and grade no.	Plot	Yield from treatment indicated			
		Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:		*Percent*	*Percent*	*Percent*	*Percent*
1 and smaller	A	20. 98	43. 70	26. 01	18. 37
	B	22. 70	13. 20	26. 20	38. 80
2	A	38. 88	40. 35	34. 11	28. 55
	B	45. 29	34. 48	36. 60	31. 40
3	A	31. 79	15. 93	30. 50	37. 52
	B	26. 20	44. 24	31. 20	21. 06
4	A	8. 36	-----	7. 48	6. 98
	B	5. 80	8. 07	5. 97	8. 74
5	A	-----	-----	1. 89	8. 58
	B	-----	-----	-----	-----
Maturity index	A	228	172	216	259
	B	215	247	217	200
Maturity index of total A and B		221	225	221	226
Harvest of June 2:					
1 and smaller	A	7. 50	10. 54	7. 04	9. 63
	B	7. 99	8. 78	5. 44	7. 61
2	A	16. 42	23. 63	11. 78	10. 81
	B	14. 80	18. 81	10. 43	24. 75
3	A	27. 72	32. 92	49. 35	53. 00
	B	45. 85	31. 20	42. 27	31. 90
4	A	35. 53	21. 69	26. 30	26. 56
	B	19. 57	29. 08	41. 85	35. 75
5	A	12. 81	11. 23	5. 55	-----
	B	11. 83	12. 09	-----	-----
Maturity index	A	330	300	312	293
	B	313	317	321	296
Maturity index of total A and B		322	310	316	296
1931 CROP					
Harvest of June 6:					
1	A	15. 71	26. 02	27. 08	15. 74
	B	17. 59	15. 08	20. 67	15. 97
2	A	39. 42	45. 56	44. 33	37. 30
	B	41. 60	43. 82	45. 44	46. 55
3	A	35. 36	24. 92	24. 19	34. 20
	B	35. 30	33. 90	29. 51	31. 94
4	A	9. 52	3. 49	4. 41	12. 76
	B	5. 49	7. 19	4. 30	5. 53
Maturity index	A	239	206	206	244
	B	229	233	217	227
Maturity index of total A and B		233	224	212	236
Harvest of June 10:					
1	A	7. 19	8. 79	8. 52	6. 48
	B	13. 30	7. 72	13. 33	12. 15
2	A	22. 75	24. 51	17. 82	19. 49
	B	31. 61	23. 52	35. 23	29. 28
3	A	38. 40	35. 82	40. 05	36. 51
	B	39. 20	42. 48	37. 72	41. 26
4	A	30. 69	29. 67	31. 15	35. 78
	B	15. 90	25. 49	13. 37	16. 74
5	A	. 96	1. 20	2. 45	1. 73
	B	. 00	. 80	. 34	. 59
Maturity index	A	295	290	301	307
	B	258	288	252	264
Maturity index of total A and B		274	289	275	283

PHYSIOLOGICAL STUDIES

Chemical analyses in connection with studies similar to those herein recorded have often been reported and discussed on the basis of oven-dry matter. Such a method of presentation has its proper applications, but alone would not be suitable here for the following reasons: (1) The study was designed to show the development and composition of fresh peas in response to certain field practices, therefore the results must be considered as they apply to fresh material; (2) peas grown in the garden or for the cannery are consumed on the fresh-weight basis, i.e., in the moisture-containing condition, so their composition as affecting nutritional value should be expressed on that basis; and (3) the results of this work when expressed on the dry-weight basis lead to conclusions for the most part opposite to, or quite different from, those reached when analyses are expressed on the fresh-weight basis. Consequently, analytical data are presented on both a fresh- and a dry-weight basis, but are discussed mainly on the fresh-weight basis. Certain facts are revealed which heretofore have not been plainly evident from the work of others who have reported their results on the basis of oven-dry matter only.

DRY MATTER

After the peas had been harvested, weighed, shelled, and graded, they were subjected to preliminary drying at 60° to 65° C. for 24 hours in the specially designed forced-draft drying apparatus already described. After drying, the samples were left for a few days in a dry room with access to the air, before they were weighed. This was done to reduce errors that might arise from the absorption of moisture during subsequent handling and grinding. The samples were ground to pass a 40-mesh sieve, and stored in tightly stoppered bottles until analyzed. The moisture content of the air-dry samples that had been dried in a preliminary way in the forced-draft apparatus was determined by drying them in an electric oven at a temperature of 103° to 105°, until constant weight was obtained. The results are recorded in table 3.

Table 3 shows that the percentage differences in the dry-matter content of pea samples grown in differently treated plots are insignificant, being mostly smaller than the percentage differences between samples from duplicate plots. For instance, the mean dry-matter percentages of grade 2, 3, and 4 peas from the untreated plot shown by samples 1, 3, and 5 and their duplicate samples 2, 4, and 6 are 22.37 and 22.87, respectively, making a difference of 0.50 percent. On the other hand, the difference between the dry-matter content of samples 2, 4, and 6 from the untreated plot (22.87) and from the potash-treated plot (23.14) is only 0.27 percent.

TABLE 3.—*Percentage of oven-dry matter in the Alaska pea as affected by different fertilizers, determined on the basis of fresh weight, 1930 and 1931*

1930 CROP

Harvest and sample no.	Grade no.	Plot	Dry matter from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	20.46	21.06	20.73	20.68
2	2	B	21.03	21.05	21.34	21.03
		Average	20.74	21.05	21.03	20.85
3	3	A	22.73	23.56	22.30	21.74
4	3	B	23.48	23.21	23.34	22.84
		Average	23.10	23.38	22.82	22.29
5	4	A	23.92		23.97	22.78
6	4	B	24.10	25.16	25.19	23.63
		Average	24.01	25.16	24.58	23.20
		Average (1–6)	22.62	22.81	22.81	22.11
Harvest of June 2:						
7	2	A	23.78	24.48	25.17	22.81
8	2	B	24.90	24.44	25.69	24.84
		Average	24.34	24.46	25.43	23.82
9	3	A	27.70	27.76	27.88	25.25
10	3	B	28.45	28.03	29.08	28.47
		Average	28.07	27.89	28.48	26.86
11	4	A	29.41	29.53	30.27	29.33
12	4	B	31.17	29.92	31.39	29.76
		Average	30.29	29.72	30.83	29.54
		Average (7–12)	27.57	27.36	28.25	26.74
		Average (1–12)	25.09	25.30	25.53	24.43
1931 CROP						
Harvest of June 10:						
13	2	A	20.15	20.08		20.94
14	2	B	21.01	21.20		20.96
		Average	20.58	20.64		20.95
15	3	A	24.89	24.81		25.57
16	3	B	25.30	26.05		25.21
		Average	25.10	25.43		25.39
17	4	A	28.57	28.11		28.83
18	4	B	27.64	28.66		29.37
		Average	28.11	28.39		29.10
		Average (13–18)	24.59	24.82		25.15

ASH CONTENT

The ashing of the pea samples was effected in an electric muffle at a dull red heat, until constant weight was obtained. The ash was white and fluffy. In no case was trickling allowed to occur. The percentages of ash in the various pea samples are recorded in table 4. In view of the importance of the ash content in foodstuffs (*5*), the following regularities are pointed out.

The ash content was greater in the riper large-sized peas than in the unripe small-sized peas from both the 1930 and 1931 crops, although it did not increase in proportion to the dry matter. This was true of all samples from a single plot at one harvest with but one exception. Another regular trend revealed by the ash results is that without exception peas of the same grade from the same plot but harvested later had a larger ash content than those harvested earlier. This increase in ash in the later harvested peas is most closely associated with increase in total dry matter; however, on the dry-weight basis the ash content ordinarily decreases with the size and age of the peas. It seems reasonable to ascribe this decrease to the fact that as the growth of the peas progresses the proportions of organic reserve substances such as carbohydrates and proteins increase more rapidly than the proportions of ash from the soil.

TABLE 4.—*Percentage of total ash in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Harvest and sample no	Grade no.	Plot	Ash from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28						
1	2	A	0.771	0.811	0.771	0.781
2	2	B	.751	.816	.785	.776
		Average	.761	.813	.778	.778
3	3	A	.816	.841	.800	.787
4	3	B	.794	.850	.822	.827
		Average	.805	.845	.811	.807
5	4	A	.854		.851	.793
6	4	B	.816	.900	.881	.827
		Average	.835	.900	.866	.810
		Average (1–6)	.800	.844	.818	.798
Harvest of June 2						
7	2	A	.855	.862	.825	.840
8	2	B	.859	.932	.860	.832
		Average	.857	.897	.842	.836
9	3	A	.942	.922	.911	.883
10	3	B	.934	1.026	.922	.922
		Average	.938	.974	.916	.902
11	4	A	.982	.978	.962	.997
12	4	B	.992	1.069	.986	.955
		Average	.987	1.023	.974	.976
		Average (7–12)	.927	.965	.911	.905
		Average (1–12)	.863	.910	.864	.851

1931 CROP, FRESH WEIGHT

Harvest and sample no	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	0.915	0.915		0.923
14	2	B	.907	.906		.959
		Average	.911	.911		.941
15	3	A	1.015	.987		1.026
16	3	B	.986	.978		1.024
		Average	1.001	.983		1.025
17	4	A	1.110	1.074		1.167
18	4	B	1.039	1.047		1.080
		Average	1.075	1.061		1.124
		Average (13–18)	.995	.985		1.030

TABLE 4.—*Percentage of total ash in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Contd.

1930 CROP, OVENDRY WEIGHT

Harvest and sample no.	Grade no.	Plot no.	Ash from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	3.77	3.85	3.72	3.78
2	2	B	3.57	3.88	3.68	3.69
		Average	3.67	3.87	3.70	3.74
3	3	A	3.59	3.57	3.59	3.62
4	3	B	3.38	3.66	3.52	3.62
		Average	3.49	3.62	3.56	3.62
5	4	A	3.57		3.55	3.48
6	4	B	3.37	3.58	3.50	3.50
		Average	3.47	3.58	3.53	3.49
		Average (1-6)	3.54	3.71	3.59	3.62
Harvest of June 2:						
7	2	A	3.60	3.52	3.28	3.68
8	2	B	3.45	3.81	3.35	3.35
		Average	3.53	3.67	3.32	3.52
9	3	A	3.40	3.32	3.27	3.50
10	3	B	3.28	3.66	3.17	3.24
		Average	3.34	3.49	3.22	3.37
11	4	A	3.34	3.31	3.18	3.40
12	4	B	3.18	3.57	3.14	3.21
		Average	3.26	3.44	3.16	3.31
		Average (7-12)	3.38	3.53	3.23	3.40
		Average (1-12)	3.46	3.61	3.41	3.51

1931 CROP, OVEN-DRY WEIGHT

Harvest and sample no.	Grade no.	Plot no.	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	4.27	4.21		4.13
14	2	B	4.06	4.02		4.32
		Average	4.17	4.12		4.23
15	3	A	3.81	3.75		3.80
16	3	B	3.69	3.57		3.88
		Average	3.75	3.66		3.84
17	4	A	3.69	3.61		8.82
18	4	B	3.54	3.46		3.53
		Average	3.62	3.54		3.68
		Average (13-18)	3.84	3.77		3.91

The difference between the ash content of peas grown in fertilized and unfertilized soil is rather insignificant, in most instances being about equal to, or even less than, the difference in the ash content of peas grown in duplicate plots. The differences in mean ash content of the phosphorus- or nitrogen-treated lots as compared with the untreated ones are certainly insignificant. It appears, however, that the potash-treated plots harvested May 28, 1930, showed a significant but small increase over the checks. In the harvests of June 2, 1930, and June 10, 1931, when the peas were at the stage usually harvested for the cannery, this difference was less striking and, indeed, was of doubtful consequence. The mean ash content of the samples from the phosphorus- and nitrogen-treated plots of this latter harvest are in remarkably close agreement with the check.

For the qualitative examination of the ash a few grams of the peas, grade 3, which were grown in soil not treated with fertilizer (table 4, no. 3, fresh-weight basis) were ashed in an electric muffle oven. The ash obtained was partly insoluble in cold and hot water but dissolved readily with the addition of a few drops of hydrochloric, nitric, sulphuric, or phosphoric acid. The ash was found to contain the following elements: Aluminum (large amount); iron, both ferric and ferrous (very little); calcium, magnesium, potassium (large amount); sodium (trace); the acids sulphuric, phosphoric (large amount), and hydrochloric (trace).

ETHER EXTRACT

Ordinarily 5- or 10-g portions of the finely ground peas were dried in an oven at 100° C. for 1 hour, after which they were transferred to fat-free paper thimbles and covered with fat-free cotton. The thimbles were than placed in the Soxhlet extraction apparatus, in which the substance was extracted with anhydrous ether for 5 hours; the ether was then driven off, the residue in the previously weighed extraction flask dried at 100° for 1 to 2 hours, cooled, and weighed.

TABLE 5.—*Percentage of ether-soluble substances in the Alaska pea as affected by different fertilizers, determined on the basis of fresh and of oven-dry weight*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Ether-soluble substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28.						
1	2	A	0.344	0.411	0.346	0.372
2	2	B	.318	.440	.414	.385
		Average	.331	.425	.380	.378
3	3	A	.382	.396	.370	.348
4	3	B	.385	.427	.387	.375
		Average	.383	.411	.378	.361
5	4	A	.335		.347	.369
6	4	B	.349	.433	.347	.359
		Average	.342	.433	.347	.364
		Average (1–6)	.352	.421	.368	.368
Harvest of June 2:						
7	2	A	.378	.392	.373	.383
8	2	B	.428	.411	.383	.405
		Average	.403	.401	.378	.394
9	3	A	.421	.419	.398	.381
10	3	B	.435	.460	.419	.407
		Average	.428	.439	.408	.394
11	4	A	.424	.446	.412	.423
12	4	B	.436	.464	.424	.432
		Average	.430	.455	.418	.427
		Average (7–12)	.420	.432	.401	.405
		Average (1–12)	.386	.427	.384	.386

TABLE 5.—*Percentage of ether-soluble substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight*—Con.

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Ether-soluble substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.388	0.361	...	0.346
14	2	B	.395	.368	...	.333
		Average	.392	.365	...	.340
15	3	A	.469	.410	...	.405
16	3	B	.433	.392	...	.375
		Average	.451	.401	...	.390
17	4	A	.493	.464	...	.458
18	4	B	.476	.472	...	.459
		Average	.485	.468	...	.459
		Average (13–18)	.442	.411	...	.396

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:						
1	2	A	1.68	1.95	1.67	1.80
2	2	B	1.51	2.09	1.94	1.83
		Average	1.60	2.02	1.81	1.82
3	3	A	1.68	1.68	1.66	1.60
4	3	B	1.64	1.84	1.66	1.64
		Average	1.66	1.76	1.66	1.62
5	4	A	1.40	...	1.45	1.62
6	4	B	1.45	1.72	1.38	1.52
		Average	1.43	1.72	1.42	1.57
		Average (1–6)	1.56	1.86	1.63	1.67
Harvest of June 2:						
7	2	A	1.59	1.60	1.48	1.68
8	2	B	1.72	1.68	1.49	1.63
		Average	1.66	1.64	1.49	1.66
9	3	A	1.52	1.49	1.43	1.51
10	3	B	1.53	1.64	1.44	1.43
		Average	1.53	1.57	1.44	1.47
11	4	A	1.44	1.51	1.36	1.44
12	4	B	1.40	1.55	1.35	1.45
		Average	1.42	1.53	1.36	1.45
		Average (7–12)	1.53	1.58	1.43	1.52
		Average (1–12)	1.55	1.70	1.53	1.60

1931 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	1.81	1.66	...	1.55
14	2	B	1.77	1.63	...	1.50
		Average	1.79	1.65	...	1.53
15	3	A	1.76	1.56	...	1.50
16	3	B	1.62	1.43	...	1.42
		Average	1.69	1.50	...	1.46
17	4	A	1.64	1.56	...	1.50
18	4	B	1.62	1.56	...	1.50
		Average	1.63	1.56	...	1.50
		Average (13–18)	1.70	1.57	...	1.50

By reference to table 5 it will be seen that, in the early stages of development, the percentages of the fat (ether extract) determined on the fresh-weight basis show no consistent relation to the grades (sizes) of the pea samples. This is probably due to differences in maturity of peas even of the same size in different plots. In the harvest of June 2, 1930, the larger sizes show, with one exception, a higher fat content than the next lower size. The same is true of the harvest of June 10, 1931. These differences are often quite small and of doubtful importance, but they are consistent.

It is evident from table 5 that peas of the same grade but harvested at a later date have, in general, a higher fat content than peas harvested earlier. Generally speaking, the percentages of ether extract parallel the percentages of ash, this being true not only of the grades but also of the age of the corresponding pea samples.

For reasons not known the fat content of the samples of grade 2 peas from the duplicate potash plot (samples 1 and 2) and the sample of one phosphorus plot (sample 2) were higher for the May 28 harvest than for the June 2 harvest. However, these 3 exceptions among 23 comparisons should not invalidate the generalization just drawn.

If the percentages of ether extract are calculated upon a dry-weight basis, all the above relationships are generally reversed, as table 5 shows.

The mean fat content of the three grades of peas from the untreated, the phosphorus-treated, and the nitrogen-treated plots for 1930 are remarkably similar for each harvest and for the two harvests combined. Appreciable differences occur between corresponding samples, but they are somewhat inconsistent. The fat content of the samples from the potash-treated plots was higher than the others in 9 out of 11 instances in 1930, but in 1931 it was lower in all 6 instances than the samples from the untreated plots, resulting in no significant [3] difference for the 2 years. In 1931 the fat content of the nitrogen-treated plots was significantly lower than the check and potash-treated plots, but the differences were quite small and unimportant. Although of doubtful consequence from a practical or culinary standpoint, this fact is interesting in a way that will be discussed later. Upon a dry-weight basis these differences are less striking than when considered as percentages of fresh weight. In 1931 the relationships just discussed for 1930 did not hold. Thus it is not possible to attach any importance to the higher fat content of the potash plots in 1930.

In practically all cases the ether extracts were found to contain not only fat but also pigments, free fatty acids, lecithin, and apparently other constituents as well. The following facts substantiate this statement. All the ether extracts were more or less colored, the color ranging from very light green or light yellow to dark yellowish green. As a rule the ether extract, at least the greater portion of it, was readily soluble in alcohol, even at room temperature. Blue litmus paper dipped into such an alcoholic solution did not change color, but when it was subsequently wet with water the color immediately changed to red. The same reaction is shown by the isolated higher fatty acids. A few crystals of chemically pure palmitic or stearic acid were dissolved in alcohol and blue litmus paper was immersed in the solution. No change of color took place; but if the litmus paper was then dipped in water, the blue color changed to red. This reaction, showing the presence of free fatty acids, was obtained with all the extracts of the various grades of peas.

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[3] As used in the discussion of results in this paper, the term "significant" or "significance" refers to differences between means for which "odds of significance" are greater than 35 to 1, by the method of Student (*1*)

Several ether extracts of the Alaska pea, after being weighed, were taken up with ether, and the ether evaporated on the water bath. The residue was boiled with barium hydroxide for about 2 hours, after which the barium salts of the fatty acids were filtered off and the filtrate evaporated on the water bath at low temperature. The residue was taken up with warm absolute alcohol, in order to remove any choline present, and the whole filtered. The residue on the filter showed the following properties· It was soluble in water, insoluble in absolute alcohol; it contained some barium and, on oxidation with a mixture of potassium nitrate and sodium carbonate, gave a slight reaction for phosphoric acid. These facts point to the presence of lecithin in the ether extracts.

While the ether extracts of the various pea samples were found to contain, in addition to glycerides, also pigments, free fatty acids, and lecithin, they differed from one another both quantitatively and qualitatively. In other words, the pea samples contained not only unequal proportions of ether extracts but they differed also in shade and intensity of color, strength of acidity, solubility, etc. For instance, unlike most of the ether extracts, two of them solidified, at least in part, and were with difficulty soluble in alcohol. The solidified substance may have consisted of wax or a waxlike matter whose nature, however, was not studied. The occurrence of waxlike substances in peas has previously been reported by Schulze and his collaborators (*25*) It is not unreasonable to assume that a careful study of the various constituents of the ether extract may throw additional light on the quality of the Alaska pea.

CARBOHYDRATES

Two grams of ground peas was transferred to a paper extraction thimble which was then covered with glass wool to prevent the substance from being thrown out of the thimble and to insure good uniform extraction. This was effected in a Soxhlet extraction apparatus by means of 60-percent alcohol, which was added to the extraction flask in a quantity sufficient to fill the extractor to the top of the siphon (usually 220 cc), leaving at the same time enough alcohol in the flask to prevent caramelization of the sugars by heat incidental to the extraction. Extraction was continued for at least 4 hours. At the expiration of this time the extract was, with the aid of hot water, transferred quantitatively to an evaporating dish and the alcohol driven off on the water bath. The alcohol-free extract was then transferred by means of hot water to a 250-cc volumetric flask, about 1 cc of 20-percent neutral lead acetate solution added for clarification, made up to the mark, and filtered until perfectly clear.

ESTIMATION OF REDUCING SUGARS

The method employed for the estimation of reducing sugars was that of Bertrand (*2*, *8*, *9*, *21*, *22*). Ordinarily a 100-cc portion of the clear filtrate was taken for this estimation. The solution was transferred to an Erlenmeyer flask of about 200- to 250-cc capacity, 20 cc of Bertrand's solution A (copper sulphate) and 20 cc of Bertrand's solution B (Rochelle salt) added, the whole brought to a boil, and the boiling continued for 3 minutes. The flask was then removed from the flame and the cuprous oxide precipitate allowed to settle well, when the supernatant blue liquid was decanted through a Soxhlet asbestos filter and sucked off. The cuprous oxide precipitate, both in the flask and on the filter, was then washed repeatedly with hot distilled water, after which the filter was removed from the suction flask, and the latter was carefully washed with water to remove any copper present. About 20 cc of Bertrand's solution C (ferric sulphate) was placed in the Erlenmeyer flask, and this dissolved all the cuprous oxide present. This solution was then poured on the Soxhlet filter, placed on the suction flask, and sucked through slowly to dissolve any cuprous oxide present on the filter. The flask was then washed with water, the wash water being used also to wash the Soxhlet filter in order to get quantitatively all the copper solution into the suction flask. This solution was then titrated with Bertrand's solution D (standard potassium permanganate) to a pink color. The results were calculated as glucose.

The potassium permanganate solution was standardized against ammonium oxalate.

Determination of Total Sugars

For the determination of total sugars 75 or 100 cc of the solution, as employed for the estimation of reducing sugars, was used. The inversion was effected by the method of Herzfeld (*7*, *10*). The solution was transferred to a 100-cc volumetric flask, 8 cc of 36-percent hydrochloric acid was added, and the whole shaken carefully. The flask with its contents was heated to 67° to 70° C. by placing it up to the neck in a water bath at 70°, this temperature being maintained for 5 more minutes Total inversion was never allowed to last more than 10 minutes. At the expiration of that time the flask was cooled with cold water, the hydrolysate neutralized with sodium hydroxide solution and made up to 100 cc. This was divided into two equal portions and employed for the determination of total sugars according to Bertrand's method as already outlined The results were calculated as invert sugar.

Estimation of Sucrose

Sucrose was calculated by subtracting the percentage of reducing sugars from that of total sugars and multiplying the difference by the factor 0.95.

Estimation of Starch

Strictly speaking, the estimation of starch deals with total acid-hydrolyzable substances rather than with starch alone, since the pea is known to contain, in addition to starch, other acid-hydrolyzable polysaccharides such as sucrose, cellulose, dextrin, hemicelluloses (xylan, araban, galactan, and mannan), and perhaps other polysaccharides and substances that yield some glucose upon hydrolysis. In this connection the following facts should be borne in mind: Sucrose has been quantitatively removed from the peas by extraction with 60-percent alcohol prior to hydrolysis; cellulose is insoluble in 2-percent hydrochloric acid used for hydrolysis of the polysaccharides; dextrin is known to occur in the pea to the extent of but 6 percent and may have been wholly or partly extracted by the 60-percent alcohol, while the exact nature and quantities of all hemicelluloses occurring in peas are not very well known. It cannot be doubted that the hemicelluloses are hydrolyzed, at least in part, under the conditions of hydrolysis as employed in this investigation. However, it seems fairly safe to state that the changes of total hydrolyzable substances give a good index to the changes in starch content. For this reason the acid-hydrolyzable polysaccharides are referred to here as starch.

The starch was estimated essentially according to the method described by Lohrisch (*17, p. 375*), Zemplén (*27*), and Schmidt (*24, p. 924*). The residue that remained from the 2 g of peas, after the sugars had been extracted with 60-percent alcohol, was transferred to a 1-l [4] flask, to which 150 cc of distilled water and 8 cc of 36-percent hydrochloric acid were added, and the mixture was hydrolyzed by boiling gently for 2 hours. The hydrolysate was then cooled, neutralized with sodium hydroxide solution, transferred to a 250-cc volumetric flask, made up to volume with water, and filtered clear. Ten-cubic-centimeter portions of this solution were used for estimating the sugar as glucose according to Bertrand's method. The starch was calculated by multiplying the glucose found by the factor 0.9. The results obtained with the various carbohydrates are summarized in tables 6, 7, and 8.

TABLE 6.—*Percentage of reducing sugars in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Reducing sugars from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.039	0.048	0.039	0.079
2	2	B	.034	.042	.041	.080
		Average	.036	.045	.040	.079
3	3	A	.064	.045	.033	.061
4	3	B	.045	.070	.037	.075
		Average	.054	.057	.035	.068
5	4	A	.048	--------	.031	.064
6	4	B	.036	.058	.030	.054
		Average	.042	.058	.030	.059
		Average (1–6)	.044	.053	.035	.069
Harvest of June 2:						
7	2	A	.071	.056	.048	.082
8	2	B	.029	.037	.044	.060
		Average	.050	.046	.046	.071
9	3	A	.042	.033	.078	.096
10	3	B	.037	.081	.047	.088
		Average	.039	.057	.062	.092
11	4	A	.050	.050	.048	.100
12	4	B	.034	.093	.041	.048
		Average	.042	.071	.044	.074
		Average (7–12)	.043	.058	.051	.079
		Average (1–12)	.044	.056	.043	.074

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[4] A large flask is indispensable, otherwise loss of liquid may occur at the beginning of boiling, when the liquid foams quite badly.

TABLE 6.—*Percentage of reducing sugars in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Reducing sugars from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.036	0.043		0.074
14	2	B	.042	.034		.084
		Average	.039	.039		.073
15	3	A	.040	.039		.057
16	3	B	.053	.060		.053
		Average	.047	.050		.055
17	4	A	.042	.059		.113
18	4	B	.059	.061		.040
		Average	.051	.060		.077
		Average (13-18)	.045	.049		.070

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of May 28:						
1	2	A	0.19	0.23	0.19	0.38
2	2	B	.16	.20	.19	.38
		Average	.18	.22	.19	.38
3	3	A	.28	.19	.15	.28
4	3	B	.19	.30	.16	.33
		Average	.24	.25	.16	.31
5	4	A	.20		.13	.28
6	4	B	.15	.23	.12	.23
		Average	.18	.23	.13	.26
		Average (1-6)	.20	.23	.16	.31
Harvest of June 2:						
7	2	A	.30	.23	.19	.36
8	2	B	.12	.15	.17	.24
		Average	.21	.19	.18	.30
9	3	A	.15	.12	.28	.38
10	3	B	.13	.29	.16	.31
		Average	.14	.21	.22	.35
11	4	A	.17	.17	.16	.34
12	4	B	.11	.31	.13	.16
		Average	.14	.24	.15	.25
		Average (7-12)	.16	.21	.18	.30
		Average (1-12)	.18	.22	.17	.31

1931 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	0.17	0.20		0.33
14	2	B	.19	.15		.38
		Average	.18	.18		.36
15	3	A	.15	.15		.21
16	3	B	.20	.22		.20
		Average	.18	.19		.21
17	4	A	.14	.20		.37
18	4	B	.20	.20		.13
		Average	.17	.20		.25
		Average (13-18)	.18	.19		.27

TABLE 7.—*Percentage of sucrose in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Sucrose from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28:						
1	2	A	4.22	3.62	3.98	4.59
2	2	B	3.55	3.61	3.89	3.93
		Average	3.88	3.61	3.93	4.26
3	3	A	3.35	2.83	3.74	4.03
4	3	B	2.72	3.03	3.31	3.53
		Average	3.03	2.93	3.52	3.78
5	4	A	3.46		3.28	3.47
6	4	B	2.53	2.88	2.87	3.02
		Average	2.99	2.88	3.07	3.24
		Average (1–6)	3.30	3.19	3.51	3.76
Harvest of June 2.						
7	2	A	4.14	3.16	3.62	4.20
8	2	B	2.90	3.83	3.29	3.57
		Average	3.52	3.49	3.45	3.88
9	3	A	2.65	2.82	3.28	3.27
10	3	B	2.41	3.13	2.94	2.55
		Average	2.53	2.97	3.11	2.91
11	4	A	2.38	2.38	2.98	3.21
12	4	B	2.43	3.21	2.56	2.57
		Average	2.40	2.79	2.77	2.89
		Average (7–12)	2.82	3.09	3.11	3.23
		Average (1–12)	3.06	3.14	3.31	3.49

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	3.42	4.11		3.86
14	2	B	3.81	3.61		4.23
		Average	3.62	3.86		4.05
15	3	A	3.08	3.08		2.91
16	3	B	2.77	2.98		3.40
		Average	2.93	3.03		3.16
17	4	A	2.54	2.55		2.22
18	4	B	2.99	2.65		3.45
		Average	2.77	2.60		2.84
		Average (13–18)	3.10	3.16		3.35

TABLE 7.—*Percentage of sucrose in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Con.

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no	Plot	Sucrose from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28:						
1	2	A	20.61	17.19	19.18	22.21
2	2	B	16.87	17.14	18.24	18.67
		Average	18.74	17.17	18.71	20.44
3	3	A	14.74	12.01	16.77	18.52
4	3	B	11.60	13.05	14.17	15.44
		Average	13.17	12.53	15.47	16.98
5	4	A	14.48		13.69	15.23
6	4	B	10.51	11.45	11.35	14.04
		Average	12.50	11.45	12.52	14.64
		Average (1-6)	14.80	14.17	15.57	17.35
Harvest of June 2:						
7	2	A	17.39	12.81	14.37	18.43
8	2	B	11.64	15.68	12.81	14.38
		Average	14.52	14.25	13.59	16.41
9	3	A	9.56	10.18	11.76	12.94
10	3	B	8.47	11.17	10.10	8.96
		Average	9.02	10.68	10.93	10.95
11	4	A	8.10	8.07	9.85	10.94
12	4	B	7.78	10.74	8.15	8.65
		Average	7.94	9.41	9.00	9.80
		Average (7-12)	10.49	11.44	11.17	12.38
		Average (1-12)	12.65	12.68	13.37	14.87

1931 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10.						
13	2	A	15.96	18.90		17.28
14	2	B	17.05	16.02		19.07
		Average	16.51	17.46		18.18
15	3	A	11.58	11.69		10.77
16	3	B	10.35	10.89		12.90
		Average	10.97	11.29		11.84
17	4	A	8.44	8.59		7.27
18	4	B	10.20	8.77		11.29
		Average	9.32	8.68		9.28
		Average (13-18)	12.26	12.48		13.10

TABLE 8.—*Percentage of acid-hydrolyzable substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Acid-hydrolyzable substances from treatment indicated: Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28:						
1	2	A	5.82	5.07	5.69	5.00
2	2	B	6.01	6.70	6.22	6.44
		Average	5.91	5.88	5.95	5.72
3	3	A	7.66	8.06	6.90	6.60
4	3	B	8.40	8.16	8.38	7.83
		Average	8.03	8.11	7.59	7.21
5	4	A	8.38	......	8.03	7.81
6	4	B	8.66	9.84	8.96	8.19
		Average	8.52	9.84	8.49	8.00
		Average (1-6)	7.49	7.56	7.34	6.98
Harvest of June 2.						
7	2	A	8 24	8.31	8.77	6.93
8	2	B	8.92	8.73	8.87	8.14
		Average	8.58	8.52	8.82	7.53
9	3	A	11.07	9.56	10.60	10.10
10	3	B	11.81	11.10	12.05	11.15
		Average	11.44	10.33	11.32	10.62
11	4	A	12.34	11.85	12.48	12.30
12	4	B	13.32	13.60	13.83	11.82
		Average	12 83	12.72	13.15	12.06
		Average (7-12)	10 95	10.53	11.10	10.07
		Average (1-12)	9.22	9.18	9.22	8.52
1931 CROP, FRESH WEIGHT						
Harvest of June 10:						
13	2	A	5.97	6.53	......	7.18
14	2	B	6.60	7.53	......	6.48
		Average	6.29	7.03	......	6.83
15	3	A	10.20	9.55	......	10.20
16	3	B	10.07	10.89	......	9.30
		Average	10.14	10.22	......	9.75
17	4	A	12.54	12.67	......	11.91
18	4	B	11.82	13.19	......	12.50
		Average	12.18	12.93	......	12.21
		Average (13-18)	9.53	10.06	......	9.60

TABLE 8.—*Percentage of acid-hydrolyzable substances in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1930 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Acid-hydrolyzable substances from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28:						
1	2	A	28.49	24.10	27.42	24.19
2	2	B	28.59	31.82	29.13	30.48
		Average	28.54	27.96	28.28	27.34
3	3	A	33.71	34.22	30.94	30.39
4	3	B	35.79	35.17	35.93	34.30
		Average	34.75	34.70	33.44	32.35
5	4	A	35.06	-------	33.52	34.30
6	4	B	35.91	39.13	35.60	34.65
		Average	35.49	39.13	34.56	34.48
		Average (1-6)	32.93	32.89	32.09	31.39
Harvest of June 2:						
7	2	A	34.65	33.97	34.84	30.39
8	2	B	35.82	35.71	34.55	32.77
		Average	35.24	34.84	34.70	31.58
9	3	A	39.99	34.43	38.01	40.00
10	3	B	41.51	39.60	41.45	39.18
		Average	40.75	37.02	39.73	39.59
11	4	A	41.94	40.11	41.21	41.96
12	4	B	42.74	45.45	44.07	39.71
		Average	42.34	42.78	42.64	40.84
		Average (7-12)	39.44	38.21	39.02	37.34
		Average (1-12)	36.18	35.79	35.56	34.36

1931 CROP, OVEN-DRY WEIGHT

Sample no.	Grade no.	Plot	Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:						
13	2	A	27.86	30.06	-------	32.16
14	2	B	29.54	33.38	-------	29.20
		Average	28.70	31.72	-------	30.68
15	3	A	38.29	36.28	-------	37.78
16	3	B	37.67	39.76	-------	35.22
		Average	37.98	38.02	-------	36.50
17	4	A	41.68	42.61	-------	38.98
18	4	B	40.26	43.59	-------	40.88
		Average	40.97	43.10	-------	39.93
		Average (13-18)	35.88	37.61	-------	35.70

DISCUSSION AND INTERPRETATION OF CARBOHYDRATE RESULTS

From table 6 it will be seen that, as a rule, the proportions of reducing sugars are almost negligible, ranging from 0.03 to 0.10 percent in the 1930 peas, and from 0.036 to 0.113 percent in the 1931 peas, calculated on the basis of fresh weight. There appears to be some difference in the percentage of reducing sugars obtained from peas grown on the variously treated plots. Thus, in the case of the 1930 peas raised in untreated soil the reducing sugars ranged from 0.029 to 0.071 percent, these extremes being duplicates; the average of all samples was 0.044 percent. Very similar results were obtained with the peas grown in phosphorus-treated soil, in which the percentage of reducing sugar ranged from 0.03 to 0.078, the average being 0.043 percent. Somewhat larger was the proportion of reducing sugars in peas from potash-treated soil, in which the percentage ranged from 0.033 to 0.093, with an average of 0.056 percent in 1930. However, the differences between corresponding samples were so inconsistent that no significance can be attached to the differences between the means just referred to. In peas from the nitrogen-treated plot the proportions of reducing sugars were slightly and significantly larger than from the check or those receiving other treatments. The analytical averages of the 1931 peas are similar to those of the 1930 peas. Considering the fact that the percentage of reducing sugars is extremely small, averaging 0.044 and 0.045 percent in peas from the untreated plot for 1930 and 1931, respectively, it would appear that there is some physiological significance in the higher percentage of reducing sugars in peas from the nitrogen-treated plot (average, 0.074 and 0.070 percent for 1930 and 1931, respectively) as compared with that in the peas from the other plots. This higher content of reducing substances, although significant statistically, is of such small magnitude as to be of no practical importance. The foregoing observations concerning determinations made on the fresh-weight basis apply equally to those made on the oven-dry weight basis.

Table 7 reveals distinct regularities in the proportions of sucrose. The figures for total sugars have been omitted, since sucrose constitutes 96 to 99 percent of the total sugars and the reducing sugars are negligible. The large-sized peas had a smaller percentage of sucrose than the small-sized peas, regardless of the time of harvest or the treatment of the plot. The older peas had a smaller sucrose content than the younger ones of the same size, age and sucrose content standing in reverse ratio.

The mean percentages of sucrose in the peas from the untreated and potash-treated plots were practically the same. The sucrose percentage in peas that received the phosphorus treatment was significantly higher than that of the check, but not significantly different from that of peas from the other plots.

The regularities in sucrose content of the peas on the fresh-weight basis hold good also for that of peas on the dry-weight basis, but the differences resulting from age and size are more striking, as will be seen by reference to table 7. That the fertilizers did not have a greater influence on the sucrose content may have been due in part to the fact that from the outset the plots were fairly fertile. At the same time it is recognized that in general it is difficult to alter the composition of seeds by these means.

Table 8 (fresh-weight basis) shows that the peas of larger grades had a correspondingly higher starch content than the peas of smaller grades, this being true of peas raised in fertilized and unfertilized plots. In other words, the starch content of the peas varied directly with their size. Another regularity noted was the higher percentage of starch in peas of the later harvest. The regularities observed in the starch content of the peas when the determinations were made on the fresh-weight basis also hold true when the starch content was determined on the oven-dry weight basis (table 8).

With regard to the influence of the various fertilizers on the starch content of the peas, table 8 shows that the 1930 peas from the untreated plot had on an average 9.22 percent of starch, those from the potash-treated plot had on an average 9.18 percent, while the average starch content of the peas from the plots treated with phosphorus and nitrogen was, respectively, 9.22 and 8.52 percent. By reducing these figures to the starch content of the peas from the untreated plot taken as 100, it will be found that the starch content of the peas from the plots treated with potash, phosphorus, and nitrogen is, respectively, 99.5, 100.0, and 92.4 percent. Thus, the nitrogen treatment has here the only significant influence on the starch formation, the effect being characteristic of delayed maturity. The difference is significant with reference to the potash- and phosphorus-treated plots as well as to the untreated plots.

The facts pointed out in the foregoing discussion will be better comprehended when the carbohydrate metabolism in peas is taken into consideration. Let us assume, as is generally assumed for plants, that the carbohydrate metabolism in peas takes place according to the following equations:

$$(1)\quad (\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O}) \longrightarrow \underset{\text{Formaldehyde}}{CH_2O} \longrightarrow \underset{\text{Glucose}}{C_6H_{12}O_6}$$

$$(2)\quad \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6} - \underset{\text{Water}}{H_2O} = \underset{\text{Sucrose}}{C_{12}H_{22}O_{11}}$$

$$(3)\quad m(\underset{\text{Glucose}}{C_6H_{12}O_6}) - n(\underset{\text{Water}}{H_2O}) = (\underset{\text{Starch}}{C_6H_{10}O_5})X$$

Then the simplest interpretation of the results obtained is that the first sugar to appear, glucose (equation 1), is rapidly changing to sucrose (equation 2). It is for this reason that the proportion of reducing sugars in the peas is quite insignificant. However, it appears that the condensation of reducing substances to sucrose and finally to starch, which is characteristic of maturity, was somewhat delayed in the peas grown on nitrogen-treated soil. This is evident from the fact that the reducing sugars in the 1930 peas from the nitrogen-treated plot (average 0.074) are significantly greater than those in peas from any of the other plots (average 0.043 to 0.056). That the nitrogen has a somewhat retarding influence on the rate of maturity of the peas is also evident from the sucrose content of the peas (table 7). The average sucrose content of the 1930 peas from the nitrogen-treated plot, the check, and the plots treated with potash and phosphorus is, respectively, 3.49, 3.06, 3.14, and 3.31. The phosphorus-treated plot produced peas of a very slightly but significantly higher sucrose content than did the check and potash-treated plots, but inasmuch as peas from the plot given the phosphorus treatment did not exhibit other differences consistently characteristic of either delayed or hastened maturity, this point is in itself unimportant. Thus, only the application of nitrogen had a definite, retarding influence on the rate of maturity of the peas, as evidenced by the sugar content. The sucrose content of the peas harvested on June 2 was smaller than that of the peas harvested on May 28 and is in full harmony with the stated metabolism in the peas.

In the later stages of development glucose is changed chiefly to starch (equation 3). For this reason the more mature peas must necessarily have a larger percentage of starch and a correspondingly smaller percentage of sucrose. That this is actually the case a glance at tables 8 and 9 will show. The fairly high percentage of sucrose and starch in peas grown in the variously treated plots is an indication that these two carbohydrates represent important reserve materials of the peas.

TOTAL NITROGEN

The total nitrogen was determined by Gunning's modification of the Kjeldahl method.

Table 9 shows that when determinations were made on a fresh-weight basis the small-sized peas as a rule had a lower percentage of nitrogen than the large-sized, and that peas harvested early had a lower nitrogen content than those harvested later. On the other hand, when determinations were made on a dry-weight basis the nitrogen content ordinarily decreased with increasing size and age of the peas.

TABLE 9.—*Percentage of total nitrogen in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*

1930 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated			
			Check	Potash	Phosphoric acid	Nitrogen
			Percent	*Percent*	*Percent*	*Percent*
Harvest of May 28:						
1	2	A	0.943	1.013	0 956	0.986
2	2	B	1.004	.967	.935	1.011
		Average	.973	.990	.945	.998
3	3	A	1.003	1.084	1.015	1.033
4	3	B	1.091	1.033	1.055	1.078
		Average	1.047	1.058	1.035	1.055
5	4	A	1.022	-------	1.054	1.041
6	4	B	1.106	1.127	1.110	1.059
		Average	1.064	1.127	1.082	1.050
		Average (1-6)	1.028	1.045	1.031	1.034
Harvest of June 2:						
7	2	A	1.010	1.092	1.102	1.052
8	2	B	1.126	1.029	1.071	1.150
		Average	1.068	1.060	1.086	1.101
9	3	A	1.169	1.235	1.185	1.119
10	3	B	1.246	1.184	1.200	1.286
		Average	1.207	1.209	1.192	1.202
11	4	A	1.215	1.329	1.286	1.296
12	4	B	1.346	1.234	1.296	1.330
		Average	1.280	1.281	1.291	1.313
		Average (7-12)	1.185	1.184	1.190	1.205
		Average (1-12)	1.106	1.121	1.105	1.119

TABLE 9.—*Percentage of total nitrogen in the Alaska pea as affected by different fertilizers; determined on the basis of fresh and of oven-dry weight, 1930 and 1931*—Continued

1931 CROP, FRESH WEIGHT

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated: Check	Potash	Phosphoric acid	Nitrogen
Harvest of June 10:			*Percent*	*Percent*	*Percent*	*Percent*
13	2	A	0.940	0.952	----------	0.990
14	2	B	.991	.981	----------	.957
		Average	.966	.967	----------	.974
15	3	A	1.156	1.126	----------	1.156
16	3	B	1.157	1.143	----------	1.111
		Average	1.157	1.135	----------	1.134
17	4	A	1.299	1.285	----------	1.302
18	4	B	1.245	1.274	----------	1.272
		Average	1.272	1.280	----------	1.287
		Average (13–18)	1.131	1.127	----------	1.131
1930 CROP, OVEN-DRY WEIGHT						
Harvest of May 28						
1	2	A	4.61	4.81	4.61	4.77
2	2	B	4.77	4.59	4.67	4.81
		Average	4.69	4.70	4.64	4.79
3	3	A	4.41	4.60	4.55	4.75
4	3	B	4.65	4.45	4.52	4.72
		Average	4.53	4.53	4.54	4.74
5	4	A	4.27	------	4.40	4.57
6	4	B	4.59	4.48	4.41	4.58
		Average	4.43	4.48	4.41	4.58
		Average (1–6)	4.55	4.59	4.53	4.70
Harvest of June 2:						
7	2	A	4.25	4.46	4.38	4.61
8	2	B	4.52	4.21	4.17	4.63
		Average	4.39	4.34	4.28	4.62
9	3	A	4.22	4.45	4.25	4.43
10	3	B	4.38	4.22	4.13	4.52
		Average	4.30	4.34	4.19	4.48
11	4	A	4.13	4.50	4.25	4.42
12	4	B	4.32	4.12	4.13	4.47
		Average	4.23	4.31	4.19	4.45
		Average (7–12)	4.30	4.33	4.22	4.51
		Average (1–12)	4.43	4.44	4.37	4.61
1931 CROP, OVEN-DRY WEIGHT						
Harvest of June 10:						
13	2	A	4.39	4.38	----------	4.43
14	2	B	4.44	4.35	----------	4.31
		Average	4.42	4.37	----------	4.37
15	3	A	4.34	4.28	----------	4.28
16	3	B	4.33	4.17	----------	4.21
		Average	4.34	4.23	----------	4.25
17	4	A	4.32	4.32	----------	4.26
18	4	B	4.24	4.21	----------	4.16
		Average	4.28	4.27	----------	4.21
		Average (13–18)	4.34	4.29	----------	4.28

There were no significant differences in the total nitrogen content of the peas from plots receiving different treatments, which is rather surprising in view of the rather marked increase of sucrose and decrease of starch in peas from the nitrogen plot.

PROTEIN AND NONPROTEIN NITROGEN

The estimation of the protein nitrogen was made according to Stutzer's method (*26*) as applied by one of the writers and reported in previous publications (*12, 13, 14, 15*). The estimation of the nonprotein nitrogen was either made directly by ascertaining the nitrogen in the filtrate from the protein precipitate as obtained by means of Stutzer's copper solution (*11, 16, 26*) or calculated by difference from 100 (tables 10 and 11).

Table 10 shows that the peas of smaller size had a smaller protein nitrogen content than those of larger size.

What is true of the relationship between the different sizes of peas is also true of the relationship between peas of different ages regardless of treatment. For instance, in 1930 grades 2, 3, and 4 of peas from the untreated plot harvested May 28 have 41.21, 48.53, and 51.29 percent of protein nitrogen, respectively (samples 1, 3, and 5), while the corresponding grades of peas harvested June 2 have higher proportions of protein nitrogen. The same holds true of the peas from the potash-, nitrogen-, and phosphorus-treated plots. The nonprotein nitrogen of peas from the various plots stands in reverse ratio to the protein nitrogen, as would be expected. Exactly the same relationships regarding size and age of the peas hold true when the percentages of protein and nonprotein nitrogen are calculated on the dry-weight basis.

The regularities herein reported regarding the decreasing sucrose content and the increasing starch and protein content with greater size and age of the peas are in harmony with the findings of other investigators (*3, 4, 23*). These facts will be better comprehended if the anabolic processes taking place in the pea are taken into account. The nitrates taken up by the pea from the soil are successively converted into nitrites, ammonia, and then into the various organic compounds according to the following scheme:

Nitrates→ nitrites→ ammonia→ mono- and di-amino acids→ acid amides→ polypeptides (peptones, proteoses) (*6, pp. 23–53*)→ proteins.

From this scheme it is plainly evident that in the earlier stages the nonproteins (amino acids, acid amides) are dominant, whereas in the latter stages the proteins, namely, legumin, vicilin, legumelin, and proteose (*18, 19, 20*) are dominant. It would seem that the regularities so conspicuously displayed by the protein nitrogen of peas from the variously treated plots, especially the very considerable and consistent differences in the protein nitrogen between peas of different sizes and of different ages, make this estimation an accurate means of determining the stage of maturity of the Alaska pea.

Although there are no significant differences in the total nitrogen content of the peas from the variously treated plots, some consistent differences occur in the distribution of the nitrogen. The mean percentage of protein nitrogen based on fresh weight of peas from the various plots for 1930 was: Check, 0.614; potash, 0.644; phosphorus, 0.627; and nitrogen, 0.599. The corresponding percentages of nonprotein nitrogen were 0.492, 0.477, 0.485, and 0.520. The differences in amount of these constituents calculated on a fresh-weight basis were too small and too variable to appear important. However, if the percentage of protein or nonprotein nitrogen is calculated on the basis of total nitrogen, some very significant differences between plots are evident. The peas from the potash plot show a protein-nitrogen content that is 56.73 percent of the total, or 1.3 times the nonprotein portion. Calculated similarly, those from the check and nitrogen-treated plots show a protein content of 54.61 and 52.66 percent, or 1.2 and 1.1 times the nonprotein-nitrogen content. The increased proportion of protein nitrogen of the potash-treated peas is not statistically significant, but the decreased proportion of the nitrogen-treated peas is significant when compared with the check or the other treatments. The peas from the phosphorus-treated plot also show a significantly higher proportion of protein nitrogen than do those of the check.

Despite the statistical significance of the differences here discussed, it is doubtful whether the magnitudes are such as to be of great practical importance from the standpoint of culinary quality. The data show, however, a tendency for potash treatment to hasten certain processes that are characteristic of maturity, and for nitrogen treatment to delay those processes.

Table 10.—*Percentage of protein and nonprotein nitrogen of the Alaska pea on fresh-weight basis and percentage of protein nitrogen based on total nitrogen, 1930 and 1931*

1930 CROP

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated											
			Check			Potash			Phosphoric acid			Nitrogen		
			Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen	Protein nitrogen		Nonprotein nitrogen
			Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis	Fresh-weight basis	Total nitrogen basis	Fresh-weight basis
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	0.389	41.21	0.554	0.431	40.75	0.600	0.407	42.52	0.549	0.391	39.62	0.595
2	2	B	.431	42.98	.573	.438	45.32	.529	.399	42.61	.536	.437	43.24	.574
		Average	.410	42.09	.563	.435	43.03	.564	.403	42.56	.542	.414	41.43	.584
3	3	A	.487	48.53	.516	.561	51.74	.523	.498	49.01	.517	.452	43.79	.581
4	3	B	.552	50.54	.539	.553	53.48	.480	.532	50.44	.523	.528	48.94	.550
		Average	.519	49.53	.527	.557	52.61	.501	.515	49.72	.520	.490	46.36	.565
5	4	A	.524	51.29	.498				.558	52.95	.496	.516	49.45	.525
6	4	B	.596	53.81	.510	.674	59.82	.453	.619	55.78	.491	.564	53.28	.495
		Average	.560	52.55	.504	.674	59.82	.453	.588	54.36	.493	.540	51.36	.510
		Average (1–6)	.496	48.06	.531	.531	50.22	.517	.502	48.88	.518	.481	46.38	.553
Harvest of June 2:														
7	2	A	.523	51.76	.487	.575	52.69	.517	.591	53.65	.511	.493	46.85	.559
8	2	B	.603	53.54	.523	.572	55.58	.457	.599	55.88	.472	.604	52.48	.546
		Average	.563	52.65	.505	.573	54.13	.487	.595	54.76	.491	.548	49.66	.552
9	3	A	.706	60.43	.463	.785	63.60	.450	.731	61.65	.454	.669	59.82	.450
10	3	B	.802	64.38	.444	.774	65.40	.410	.799	66.59	.501	.806	62.61	.480
		Average	.754	62.40	.453	.779	64.50	.430	.765	64.12	.477	.737	61.21	.465

11	4	A	806	66 34	409	880	66 22	449	881	68 24	.405	851	65.61	.445
12	4	B	.951	70 60	395	857	69 42	377	.917	70 70	379	881	66 22	.449
		Average	.878	68 47	402	868	67 82	.413	899	69.47	392	866	65.91	.447
		Average (7–12)	.732	61.17	.453	740	62 15	443	753	62.78	453	.717	58.94	.488
		Average (1–12)	614	54 61	.492	644	56 73	477	627	55.83	485	.599	52.66	.520
1931 CROP														
Harvest of June 10:														
13	2	A	0 446	47 38	0 495	0 448	47 03	0 504				0 474	47 86	0.516
14	2	B	476	47 97	.516	489	49 89	.492				.444	46 40	.513
		Average	.461	47 68	506	469	48 46	498				.459	47 13	515
15	3	A	.719	62.21	437	687	60.98	439				.689	59.58	.467
16	3	B	.724	62 59	.433	726	63 55	.416				.657	59.14	.454
		Average	722	62 40	435	707	62 27	428				673	59.36	.461
17	4	A	.899	69 21	400	.880	68 52	404				.892	68.54	.409
18	4	B	866	69 58	379	908	71 26	.366				859	67.55	.411
		Average	.883	69 40	.390	894	69 89	.385				876	68 05	.413
		Average (13–18)	.688	59 82	443	690	60 21	437				.669	58.18	.462

TABLE 11.—*Percentage of protein and nonprotein nitrogen of the Alaska pea on oven-dry weight basis and percentage of nonprotein nitrogen based on total nitrogen*

1930 CROP

Sample no.	Grade no.	Plot	Nitrogen from treatment indicated											
			Check			Potash			Phosphoric acid			Nitrogen		
			Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen		Protein nitrogen	Nonprotein nitrogen	
			Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis	Oven-dry weight basis	Oven-dry weight basis	Total-nitrogen basis
Harvest of May 28:			*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	2	A	1.90	2.71	58.79	1.96	2.85	59.25	1.96	2.65	57.48	1.89	2.88	60.38
2	2	B	2.05	2.72	57.02	2.08	2.51	54.68	1.99	2.68	57.39	2.08	2.73	56.76
		Average	1.98	2.72	57.91	2.02	2.68	56.97	1.98	2.67	57.44	1.99	2.81	58.57
3	3	A	2.14	2.27	51.47	2.38	2.22	48.26	2.23	2.32	50.99	2.08	2.67	56.21
4	3	B	2.35	2.30	49.46	2.38	2.07	46.52	2.28	2.24	49.56	2.31	2.41	51.06
		Average	2.25	2.29	50.47	2.38	2.15	47.39	2.26	2.28	50.28	2.20	2.54	53.64
5	4	A	2.19	2.08	48.71				2.33	2.07	47.05	2.26	2.31	50.55
6	4	B	2.47	2.12	46.19	2.68	1.80	40.18	2.46	1.95	44.22	2.44	2.14	46.72
		Average	2.33	2.10	47.45	2.68	1.80	40.18	2.40	2.01	45.64	2.35	2.23	48.64
		Average (1-6)	2.18	2.37	51.94	2.30	2.29	49.78	2.21	2.32	51.12	2.18	2.52	53.61
Harvest of June 2.														
7	2	A	2.20	2.05	48.24	2.35	2.11	47.31	2.35	2.03	46.35	2.16	2.45	53.15
8	2	B	2.42	2.10	46.46	2.34	1.87	44.42	2.33	1.84	44.12	2.43	2.20	47.52
		Average	2.31	2.08	47.35	2.35	1.99	45.87	2.34	1.94	45.24	2.30	3.33	50.34
9	3	A	2.55	1.67	39.57	2.83	1.62	36.40	2.62	1.63	38.35	2.65	1.78	40.18
10	3	B	2.82	1.56	35.62	2.76	1.46	34.60	2.75	1.38	33.41	2.83	1.69	37.39
		Average	2.69	1.62	37.60	2.80	1.54	35.50	2.69	1.51	35.88	2.74	1.74	38.79

11	4	A	2.74	1.39	33.66	2.98	1.52	33.78	2.90	1.35	31.76	2.90	1.52	34.39
12	4	B	3.05	1.27	29.40	2.86	1.26	30.58	2.92	1.21	29.30	2.96	1.51	33.78
		Average	2.90	1.33	31.53	2.92	1.39	32.18	2.91	1.28	30.53	2.93	1.52	34.09
		Average (7-12)	2.63	1.67	38.83	2.69	1.64	37.85	2.65	1.57	37.22	2.66	1.86	41.07
		Average (1-12)	2.41	2.02	45.38	2.51	1.94	43.27	2.43	1.95	44.17	2.42	2.19	47.34
1931 CROP														
Harvest of June 10:														
13	2	A	2.08	2.31	52.62	2.06	2.32	52.97				2.12	2.31	52.14
14	2	B	2.13	2.31	52.03	2.17	2.18	50.11				2.00	2.31	53.60
		Average	2.11	2.31	52.33	2.12	2.25	51.54				2.06	2.31	52.87
15	3	A	2.70	1.64	37.79	2.61	1.67	39.02				2.55	1.73	40.42
16	3	B	2.71	1.62	37.41	2.65	1.52	36.45				2.49	1.72	40.86
		Average	2.71	1.63	37.60	2.63	1.60	37.74				2.52	1.73	40.64
17	4	A	2.99	1.33	30.79	2.96	1.36	31.48				2.92	1.34	31.46
18	4	B	2.95	1.29	30.42	3.00	1.21	28.74				2.81	1.35	32.45
		Average	2.97	1.31	30.61	2.98	1.29	30.11				2.87	1.35	31.96
		Average (13-18)	2.59	1.75	40.18	2.58	1.71	39.80				2.48	1.79	41.82

SUMMARY AND CONCLUSIONS

The application of superphosphate at the rate of 1,000 pounds per acre (160 pounds phosphoric acid) produced a definite increase in yield of the Alaska pea over the untreated plot, amounting to 24 and 18 percent (fresh weight) for the seasons of 1930 and 1931, respectively. On the other hand, the application of 300 pounds of muriate of potash per acre (144 pounds potash) and of 400 pounds nitrate of soda plus 280 pounds of sulphate of ammonia (116 pounds nitrogen) gave differences in yield, as compared with the untreated plot, which were neither sufficiently great nor sufficiently consistent to permit the drawing of definite conclusions.

Records of direct observations in the field, as well as the very similar maturity indices for the peas from the treated and untreated plots, do not show any perceptible consistent differences in the rate of maturity. This is in harmony with earlier field observations and with reports of other investigators as well as with the chemical analyses of the peas from plots treated with potash and phosphorus. However, the peas from the nitrogen-treated plot showed, on an average for the entire season, a somewhat higher percentage of reducing sugars, a significantly higher percentage of sucrose, and a distinctly lower starch content as compared with the peas from the untreated plot.

Although the various treatments were without consistent effect on content of total nitrogen, the peas of the nitrogen-treated plot exhibited a slightly but significantly smaller proportion of protein nitrogen than peas from the check or any of the other plots. On the other hand, peas grown on the potash-treated and phosphorus-treated plots showed a larger proportion of protein than did the check, suggesting more advanced or hastened maturity as a result of the treatments. However, the phosphorus treatment was followed by no noticeable difference in percentage of any of the carbohydrates, of ash, or of ether extract. Thus, it seems safe to conclude that the maturity and hence the quality of the peas were unaffected by the superphosphate. The peas from the potash plots, on the other hand, exhibited slightly and significantly higher ash and ether-extract content than did the checks and those receiving the other treatments. Potash treatment was accompanied by increases in 3 of 5 constituents which are known to increase with maturity. Only the two carbohydrate fractions, sucrose and starch, which are believed to be of primary importance in determining quality and maturity, were unaffected. Thus it seems that potash does have a slight tendency to hasten the maturity of the pea. This tendency, however, is so very slight, the differences are of such very small magnitude, that they are of questionable practical importance.

Stages of maturity are characterized by distinct and marked differences. In general, more mature peas, i.e., larger peas or peas of the same size but of later harvest, have a larger percentage of ash, of ether extract (fat), total nitrogen, starch, and protein nitrogen, and a lower percentage of sucrose. On a dry-weight basis the percentages of all constituents except starch and protein nitrogen decrease as maturity progresses.

Muriate of potash and superphosphate applied singly are apparently without consistent and significant effect upon the rate of development or the quality of Alaska peas as indicated by partial chemical analysis or behavior in the field. Readily available nitrogen tends to delay maturity appreciably, as is indicated by the higher sugar and lower protein and hydrolyzable-polysaccharide content.

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ABNORMALITIES IN THE FLOWER AND FRUIT OF CAPSICUM FRUTESCENS[1]

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INTRODUCTION

Relatively few cases of abnormalities in *Capsicum frutescens* have received attention in the literature. The cases reported are scattered over a period of more than half a century. No detailed historical study has apparently been made to differentiate between abnormalities infrequently occurring in the flower and the normal flower itself, and only in the paper by Harris (*6*)[3] has any such work been reported on those more frequently found in the fruit. It is the object of this paper, therefore, to review the literature confined to abnormalities in the genus *Capsicum*, and to present a more detailed study of those occurring in both the flower and the fruit.

The work reported here was done in connection with some studies that are being carried out on fruit setting in the pepper (*Capsicum frutescens* L.) as influenced by certain environmental factors.

LITERATURE REVIEW

The earliest and yet perhaps the most extensive investigation concerning abnormalities in the genus *Capsicum* is that of Terracciano (*15*), who noted the changing of stamens into carpels and a growing together of these with the pistil in the flower of *C. grossum*. He also reported two types of abnormalities in the fruit of *C. annuum*. In one form the walls of the fruit, terminated by a hollow tubiform style open at the top, showed five protuberant evaginations. Internally the fruit produced no seeds but contained five similar fruits, likewise without seeds and with short styles having almost perfect stigmas, disposed upon the apex of the thalamus.

Mottareale (*10*) reported adesmy of the carpels in several cases, and stamens that were both larger and smaller than the corolla pieces. He also saw diaphysis and ecblastesis of the fruit. Both abnormalities were in a few cases seen in the same fruit. Mottareale cites Terracciano as having established that the staminate vasal collar develops externally like the corolla and is capable in *Capsicum grossum* of generating carpels. Not only are the carpels formed at the alternating petalous points, transformation of the normal stamens, but also at the epipetalous points, forming small fruits by development and also by a change of the basal teeth with which every stamen is provided.

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[3] Reference is made by number (italic) to Literature Cited, p 747.

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Heckel (*8*) observed pistillody of the stamens and a coalescence of them with the pistil in flowers of *Capsicum annuum*. He also reported that abnormalities in the fruit were relatively common. He found only a single carpel in some, while in others there were present more or less well formed fruits.

Some unusual types of abnormalities have been reported, chief among which is one by Schilberszky (*13*). From a common pedicel two individual fruits of *Capsicum annuum* arose and were grown together at the lower side in an angle of about 80°. Both fruits were equal in size and normally developed. The calyx which usually has 5 points, in this case had 10 and was forced between the single fruits in such a manner that it formed an obtuse angle.

Gallardo (*2*) has described a case in *Capsicum annuum* where internal tuberlike formations as large as 2.5 cm in diameter appeared, which he thought resulted from seeds becoming swollen. Welter (*17*) found that very often the seeds of *C. annuum* sprouted in the fruit.

Viviand-Morel (*16*) reported a small internal fruit in the pepper which originated from the apex of the axis, while Halstead (*4*, *5*) noted an almost identical case of a miniature included fruit that arose from the apex of the fleshy columella on which the seeds are borne. It resembled a normal fruit very much and was green. Raymondaud (*12*) also noted a small, globular, olive-shaped internal fruit in *Capsicum annuum*.

Penzig (*11, v. 2, p. 174*) reported, after a long study, that abnormalities occurred rather frequently in the fruit of the genus *Capsicum*. Penzig (*11, v. 3, p. 77*) cites Borbás (*1*) as having found central prolification of the fruit in the same genus. This is also borne out by the findings of Harris (*6*). Irish (*9*) in his extensive work with the pepper has reported similar abnormalities in the fruit.

Sturtevant (*14*) reported that sweet peppers were subject to the development of a berry or berries within the berry. These enclosed fruitlike bodies were in some cases found to produce a few seeds.

MATERIALS AND METHODS

During 1932–33 two crops of peppers were grown in the experimental greenhouses of the Department of Vegetable Crops at Cornell University. Plants from which material was collected were of the World Beater variety grown both in bank sand, to give a low-nitrogen series, and in soil high in organic matter to which sodium nitrate was added at weekly intervals to give a high-nitrogen series, in 1-gallon glazed crocks. One plant was placed in each crock and grown under two controlled temperature conditions, medium 60°–70° F., and warm 70°–80°. Fresh material was observed under a wide-field binocular microscope. Slides were made of paraffin sections, the material being killed and fixed in a solution made as follows: Solution A, 1 g chromic acid and 10 cc glacial acetic acid in 90 cc of water; solution B, 40 cc of commercial 40 percent formalin added to 10 cc of water (Karpechenko's chromo-acetic). Equal parts of the two solutions were mixed at the time they were used. Heidenhain's iron-alum haematoxylin was found very satisfactory for staining all tissues studied in the histological part of the investigation.

TERATOLOGICAL TRANSITIONS IN THE FLOWER

The normal flower of *Capsicum frutescens* (fig. 1, *D*) occurs either singly or 2 or 3 together in the axils of branches. It has a minutely 5-lobed calyx and a rotate corolla that is also 5-merous. The syle is linear, straight, longer than the stamens, and terminated by a subcapitate stigma. There are 5 stamens attached to the ovary near the base of the corolla having heart-shaped anthers that dehisce by longitudinal splitting. The ovary is usually 3-celled.

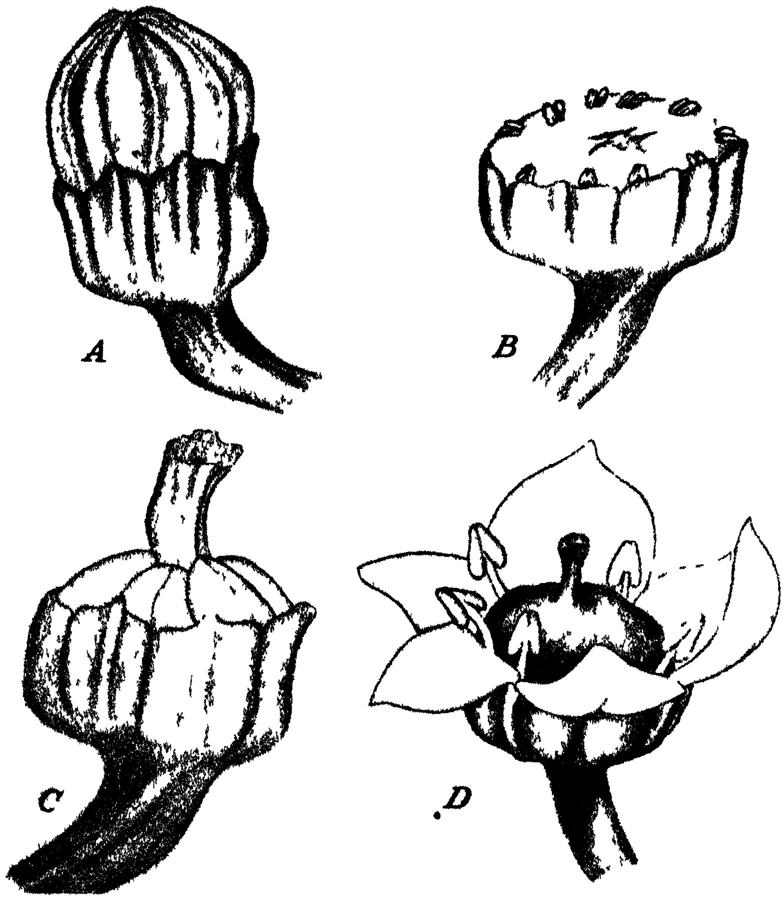

FIGURE 1.—*A*, Normal bud 1 day prior to anthesis; *B*, abnormal bud devoid of pistil (note contorted area where pistil usually is attached) and stamens devoid of filaments; *C*, young abnormal bud with protruding style, to be compared with *A*; *D*, normal flower on date of anthesis

In the case of the abnormal flower (fig. 2, *A*) which is ordinarily easily recognized, some interesting teratological changes sometimes take place. The calyx may undergo partial metamorphosis, thus exhibiting almost unconceivable transitions. One very abnormal case was noted in this study when a calyx lobe enlarged and assumed the appearance of an almost fully developed petal (petalody), while another calyx lobe on the same flower developed into a short pistil (pistillody). According to Gray (*3, p. 174*) pistillody from parts other than the stamens rarely occurs. Some abnormal flowers have normal calyxes, i.e., 5-lobed, others have as many as 8, while still others, as was noted by Mottareale (*10*) and the writer, may be completely deprived of the calyx.

A great variation was found in the stage of development of the flower at which the transformations occur as well as the time taken for their completion. Staminody may be initiated in the young bud stage and completed during that time, or it may not start until about the time of anthesis. The abnormal phenomenon may cease at this stage, leaving a contorted-appearing organ or after a time complete itself.

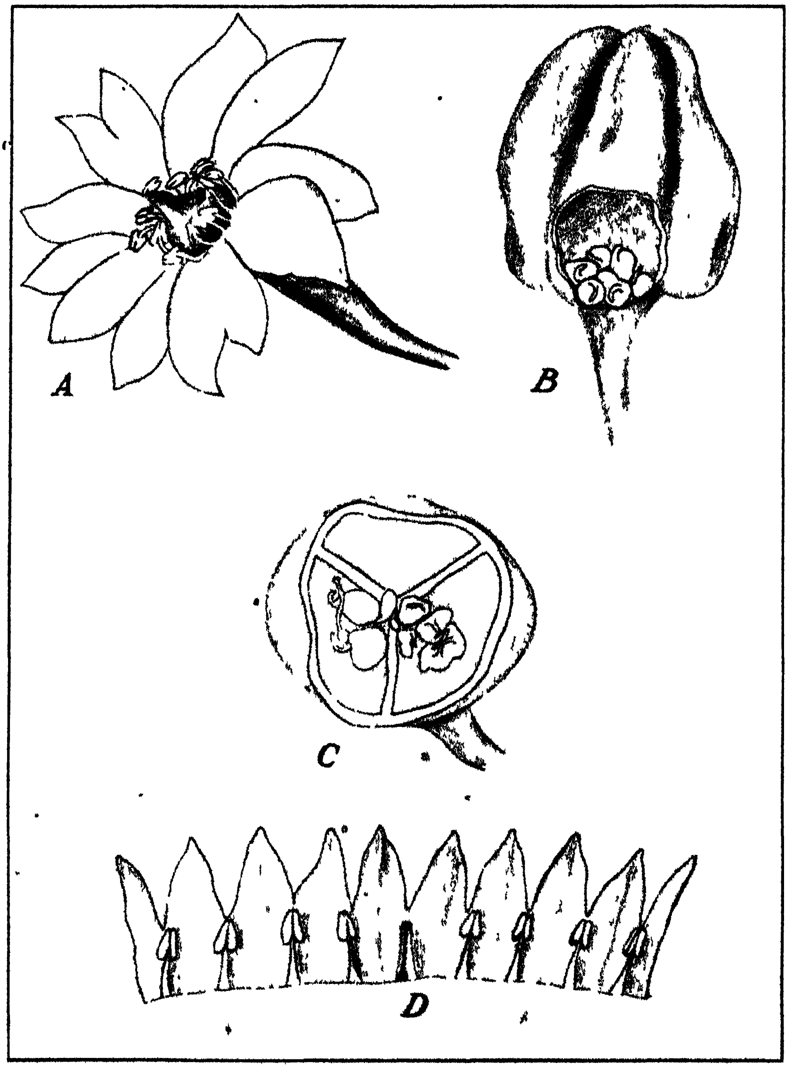

FIGURE 2.—*A*, Abnormal flower with many petals, some divided, and 13 stamens; *B–C*, mature fruits showing internal fruitlike bodies on receptacle; *D*, abnormal corolla with 9 stamens attached, of which 8 are normal, and 1 lacks the anther, but has instead a round hole extending to bottom of filament.

As is shown in figure 3 the abnormal corolla does not shed within 2 or 3 days or become withered, like that of the normal flower. It persists until the ovary develops to the point that the basal collar is split, thus allowing the corolla to drop.

While the majority of abnormal flowers contain from 7 to 9 stamens, occasionally there may be more. In figure 2, *A* is shown a flower with 13 normal stamens, whereas in figure 1, *B*, is shown another flower with 10 anthers completely deprived of their filaments. In figure 2, *D*, is a flower with 9 stamens, 8 normal ones and another devoid of its anther but with an opening at the apex that extends to the bottom of the filament. A similar case of the latter abnormality was reported by Mottareale (*10*).

Stamens sometimes undergo partial retrogressive metamorphosis thus causing some of them to unite with the petals, and they may even take the appearance of petals but may not complete the transition to petalody. However, this completion was observed during the course of this study.

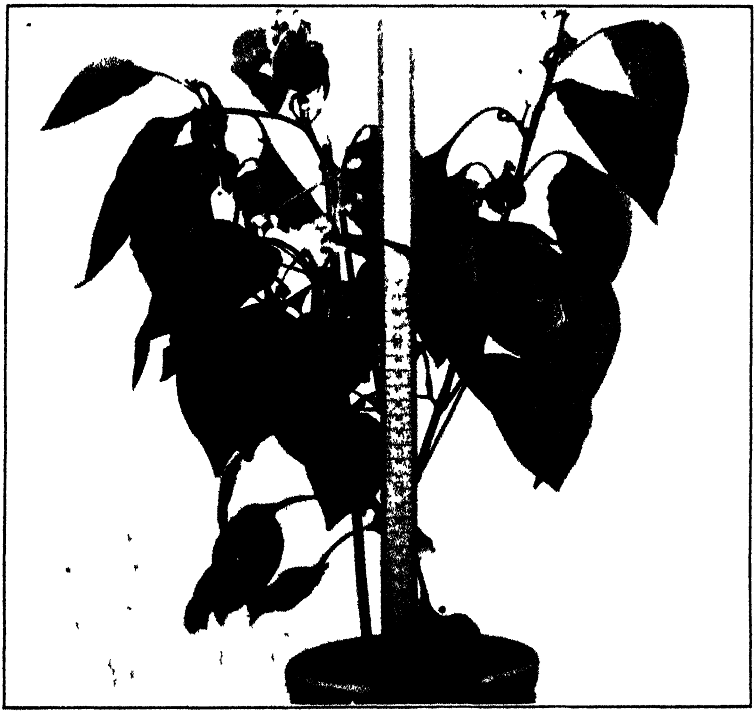

FIGURE 3.—Plant of *Capsicum frutescens* showing abnormal corollas which persist in being attached

The abnormal style is perhaps the most easily recognized flower part. Normally it can be detected in the early bud stage by its unusual protrusion above the tightly closed petals. It is generally short and broad, and often severely contorted as is shown in figure 1, *C*. Complete anthesis of such flowers is delayed, and owing to the early emergence of the stigmatic surface beyond the stamens, the chances for self-pollination are very small. Owing to this and to the fact that sometimes such stigmatic surfaces fail to become receptive, the ovules are not fertilized and the fruits develop parthenocarpically.

However, cross-pollination and perhaps self-pollination, evidently take place in a few cases. A point of great interest at this stage is the behavior of such styles with reference to their development and abscission. Normal styles at the time of anthesis are rarely more than 1 mm in diameter near the base and 5 mm in length. The styles usually dry up and drop soon after fertilization has taken place. Very often the style of an abnormal flower has become 2 to 2.5 mm in diameter and 7 to 9 mm in length by the time the flower opens. It does not absciss but invariably remains attached to the ovary, develops chlorophyll, and increases in size with the ovary until maturity, at which time it takes on the normal red color. The development of the various stages of the abnormal style is shown in figure 4. Occasionally flowers develop devoid of styles as can be seen in figure 1, *B*. There is usually a more or less contorted cavity at the apex of the ovary in such fruits. The ovary may in some cases split longitudinally into a rosette of stylar portions, some separate, other united. Each portion may develop a stigmatic surface which appears to be very abnormal. Such conditions are not found very frequently, however, and the stage to which they develop depends on the age of the ovary

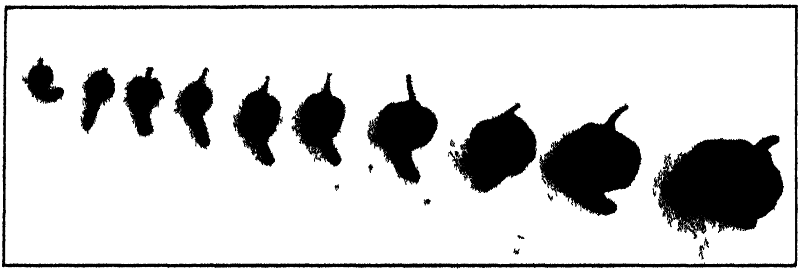

FIGURE 4.—Development of the abnormal style It does not absciss but continues in growth with the ovary Corollas have been removed from the larger fruits

TERATOLOGICAL TRANSFORMATIONS IN THE FRUIT

Harris (7) in working with okra, states:

> Among the various phenomena that may be included by the teratologists under the term "prolification of the fruit", one of the most interesting is the production of a more or less completely formed second fruit inside the first Generally, the included "fruit" is distinctly abnormal in character, often reduced to a whorl, or a series of whorls, of irregularly formed and usually sterile carpels

The fruitlike structures in *Capsicum frutescens* apparently have no definite form but vary from an irregular contorted body through an almost perfectly formed sterile fruit, comparable in shape with the one in which it is produced, to linear bodies from a few millimeters to 2 cm in length, some of which are terminated by a minute style A few of the fruitlike bodies noted, however, contained no styles, as is shown in table 1 It is also apparent from these data that other bodies contained more than one style. Several bodies had as many as three styles, and they were not always normal in form as some were very much fasciated In one case there were four styles. One style, however, was practically always found attached at the apex of the body while the others were usually formed near the apex or on the side. This is shown in figure 2, *B*, *C*.

TABLE 1.—*Distribution of abnormalities in the fruits of Capsicum frutescens*

Lot no.	Date fruits were harvested	Temperature under which plants were grown	Fruits harvested	Normal fruits	Abnormal fruits	Fruits abnormal	Internal abnormalities	Abnormalities having styles	Styles contained	Seeds in abnormal fruits
		° F	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*
1	Dec. 7, 1932	60-70	191	188	3	1 57	71	50	76	306
2	Dec. 26, 1932	70-80	129	123	6	4. 65	55	42	78	14
3	Jan. 11, 1933	70-80	213	204	9	4 22	69	43	54	34
4	Jan. 21, 1933	60-70	175	170	5	2 85	33	25	61	1[illegible]
5	Feb. 4, 1933	70-80	163	156	7	4 29	37	28	54	2[illegible]
6	Feb. 16, 1933	60-70	228	217	11	4 82	34	16	32	28
7	Feb. 28, 1933	60-70	195	187	8	4 10	22	15	21	95
8	Mar 19, 1933	70-80	142	132	10	7 04	36	19	32	1
9	May 6, 1933	70-80	223	216	7	3. 13	15	10	21	280
10	May 19, 1933	([a])	216	202	14	6. 48	50	40	86	9
11	July 13, 1933	([a])	147	125	22	14 96	55	41	81	75
Total			2, 022	1, 920	102	5 04	477	329	596	886

[a] Temperature uncontrolled

The data in table 1 show a seasonal distribution of the abnormalities in the fruits. Even though the percentage of abnormal fruits was not unusually high at any one time, abnormal fruits were present throughout the duration of the experiment. The number of internal bodies was in no way constant. In fact, the determination of such was sometimes rather difficult because of the varying degrees of fusion of the members of the outer whorls and the small size of those of the inner ones. Harris (*6*) counted the number of styles and used this as a criterion of the number of internal abonormalities in *Passiflora*, but the writer found the method unsatisfactory in the present study because some of the included bodies contained more than one style. In the majority of cases reported here, counts were made under a wide-field binocular microscope using a dissecting needle to separate the sometimes much entangled fruitlike structures. Some fruits contained only 1 of these structures, while others had as many as 18 to 20, and in one case 22 of such abnormalities were noted. They completely filled the fruit cavity.

HISTOLOGICAL DIFFERENTIATIONS BETWEEN PARTS OF THE NORMAL FLOWER AND FRUIT AND EXISTING ABNORMALITIES

Since no previous work has been reported that differentiates histologically between abnormalities infrequently occurring in the flower and the normal flower itself, attention has naturally been focused on this relationship here. Figure 5, *A* and *B*, shows cross sections of a normal style and the style of an internal fruitlike body. Both are similar in some tissues, yet as a whole their development and tissue arrangement resemble each other but very little. The style of the abnormal fruit failed to develop a stylar canal. This fact may offer a partial explanation for the failure of the internal abnormal fruitlike bodies to become fertilized, and to form seed even when pollen has been applied to the stigmalike surface. Some abnormal styles were found to contain only 1 vascular bundle while others were frequently noted that contained from 6 to 8. The usual number of vascular bundles found in the normal style is 4.

The conducting tissue through which the pollen tubes pass is usually found immediately surrounding the stylar canal as is shown in figure 5, *A* In the abnormal style it may be completely absent or found to one side and sometimes mixed in with the parenchyma tissue. This can be seen in figure 5, *B*

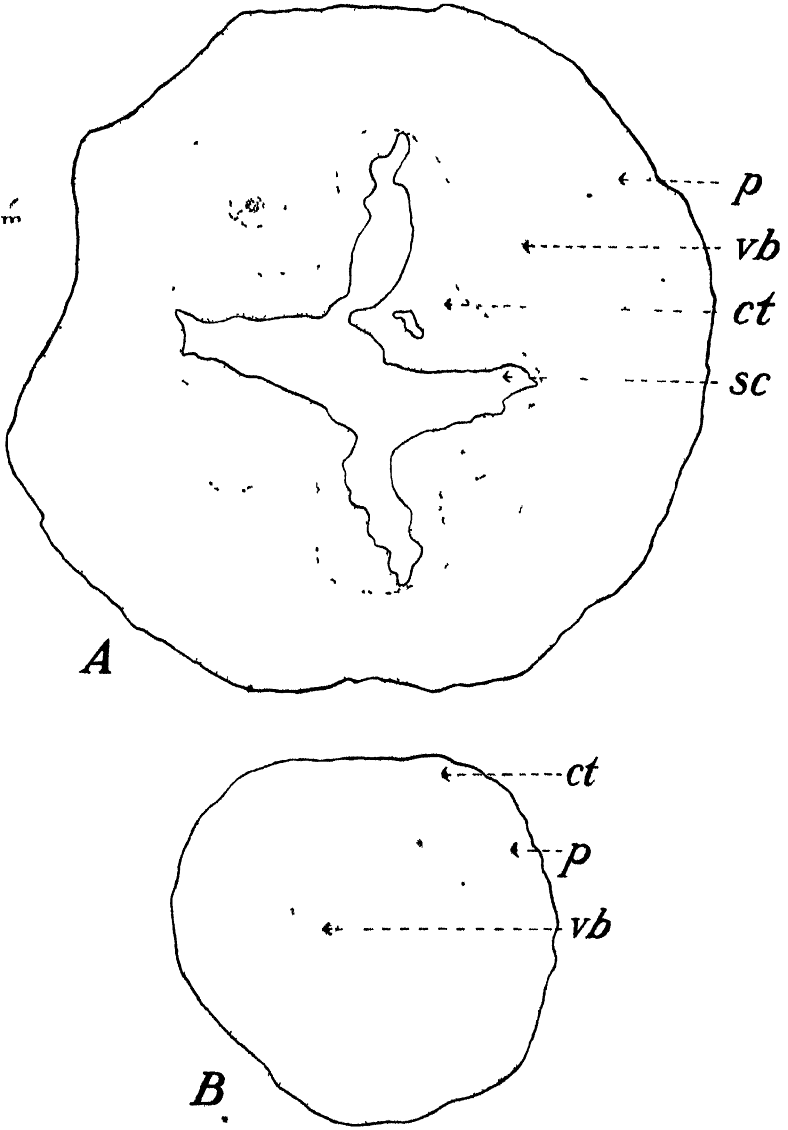

FIGURE 5 *A*, Cross section of normal style *p*, Parenchyma *vb*, vascular bundle, *ct*, conducting tissue *sc*, stylar canal *B*, Cross section of abnormal style of internal fruitlike body ×178

Harris, (*6*, *p. 137*) in his mention of the histological similarity existing between carpellike bodies and the wall of the fruit, writes:

> Both show the exceedingly heavy epidermis and the large-celled parenchymatous ground tissue. The bodies show one or more vascular bundles similar to

those of the wall of the fruit. When fresh sections are examined, numerous chloroplasts are seen in both but they are generally more abundant in the wall of the fruit.

That the wall of the internal fruitlike body is similar histologically to the wall of a normal fruit is shown in figure 6, *A* and *B*. There are some differences noted, however, in that the wall of the fruit contains several layers of cells constituting the epicarp which are absent in the wall of the fruitlike body. The former also contains much larger

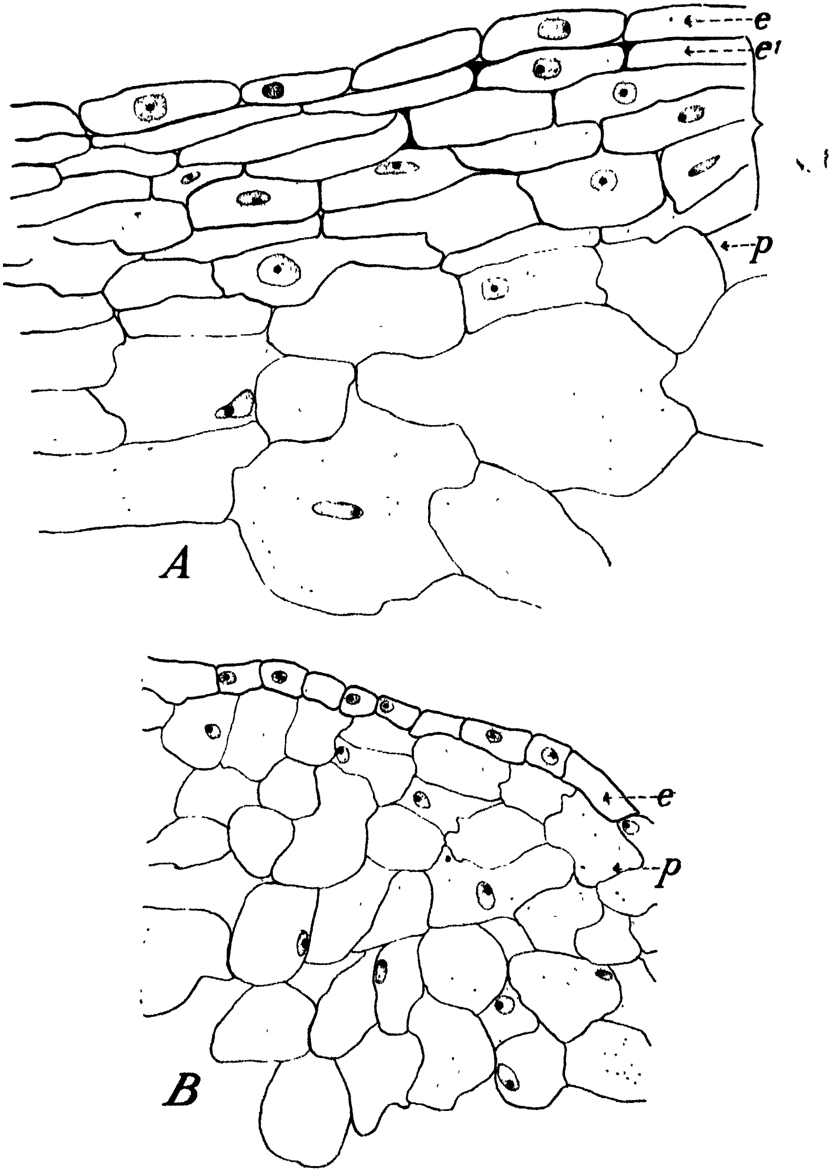

FIGURE 6.—*A*, Cross section of wall of normal fruit: *e*, Epidermis; *e'*, epicarp; *p*, parenchyma *B*, Cross section of wall of internal fruitlike body. × 750.

parenchyma cells than does the latter. The chloroplasts and vascular bundles were similar in both cases.

DISCUSSION

The question as to what factors cause the development of abnormalities has probably arisen in the minds of all workers who have reported them, but up to the present time explanations are lacking. Mottareale (*10*) is the only previous worker who has attempted to correlate environmental conditions with the existence of these abnormalities. He attributed the abnormalities found in the flower and fruit of *Capsicum annum* and *C. grossum* to short periods of cold weather under which the plants were grown. This explanation, however, does not seem altogether feasible, since he later repeated the same treatment twice and was unable to obtain similar results. Work of the writer tends to disprove the idea that low temperature is the only factor that results in the initiation and production of abnormalities. Those that are reported here were produced on plants grown under controlled medium (60° to 70° F.) and high (70° to 80°) temperatures in greenhouses during the winter of 1932, and under uncontrolled temperatures (70° to 110°) in greenhouses during the summer of 1933. This does not prove, however, that abnormalities would not be initiated under still lower temperatures than those used in this study. The data in table 1 show that the number as well as the percentage of abnormal fruits was greater during the summer under high uncontrolled temperatures than at any other time of the year. This fact suggests that high temperature is more effective in producing abnormal fruits than is medium temperature. In addition, the results of examination of a large number of flowers during the same periods agree with these findings in regard to the fruits.

From the beginning of this study indications seemed to show that a competition for nutrients or elaborated food between the rapidly growing normal plant parts may be the cause of the initiation of abnormalities since they appeared first on plants in the low-nitrogen series. Soon afterwards, however, several abnormal fruits and one abnormal flower were noted on plants in the high-nitrogen series. In fact, by the time the experiment terminated plants in the high-nitrogen series had produced more abnormalities than those in the low-nitrogen series, probably because the former produced more flowers and fruits.

SUMMARY

A review of the literature concerning abnormalities in the genus *Capsicum* and a detailed study of those occurring in both the flower and the fruit are reported here.

A very rare case was noted in which two calyx lobes on the same flower assumed different teratological transformations, namely, pistillody and petalody.

There is no definite stage in the formation of the flower at which the transitions are initiated or completed.

The abnormal corolla does not drop or wither within 2 or 3 days but persists until the ovary develops to the point that the basal collar splits and this allows the corolla to drop.

Stamens may undergo partial retrogressive metamorphosis, thus causing some of them to unite with the petals, and may even take

the appearance of such, but may not complete the transition to petalody.

The abnormal style can commonly be detected in the early bud stage by its unusual protrusion above the tightly closed petals. It does not absciss but remains attached to the ovary, develops chlorophyll, and increases in size with the ovary until maturity, at which time it takes on the normal red color.

The internal fruitlike bodies are usually distinctly abnormal in character. Some have no style, while other have as many as 3, and in one case 4 were noted.

The number of internal abnormalities was in no way constant, as they ranged all the way from 1 to 22.

Histological differentiations are made between parts of the normal flower and fruit and existing abnormalities.

Both the style of a normal flower and that of an interal fruitlike body are similar in some tissues, yet as a whole their development and tissue arrangement parallel each other but very little. The fruitlike bodies failed to develop any indications of a stylar canal.

Histological similarities between the wall of the internal fruitlike body and the wall of the fruit are interesting. Both have a heavy epidermis, large parenchyma cells, chloroplasts, and vascular bundles. There is one very distinct difference, however, in that the fruit wall contains several layers of cells, which make up the epicarp and which are absent in the wall of the carpel like body.

While both abnormal flowers and fruits were produced more abundantly on plants growing under high and high uncontrolled temperatures, they appeared to some extent on plants under medium temperature.

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SOIL TREATMENT IN RELATION TO CLUBROOT OF CABBAGE[1]

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INTRODUCTION

The investigation herein described is a continuation of work carried on by Wellman[2] during the years 1924 to 1928 inclusive. In a report of his studies he noted that the hydrate was the most effective compound of calcium used to control clubroot of cabbage. In 1928 he secured good control of the disease on severely infested soil in Kenosha County, Wis., with a 2-ton treatment of this chemical. In 1930 and 1931 field treatments were carried out by the writers on infested soil in the same locality. Since in these cases even heavier treatments of the soil with calcium hydrate had little effect upon clubroot infection, further studies were undertaken to determine, if possible, what factors might influence the effectiveness of liming materials.

Previous work on the relation of soil reaction to infection by the clubroot organism (*Plasmodiophora brassicae* Wor.) has been reviewed by Wellman. Chupp[3] and Wellman[4] both succeeded in completely inhibiting infection in the greenhouse by applying calcium hydrate in sufficient quantities to make the soil reaction slightly alkaline. In the field experiments results were not always consistent. Ravn[5] secured considerable reduction in infection by the use of finely divided $CaCO_3$ and still better control with a mixture of $CaCO_3$ and CaO. It is important to note, however, that in some cases severe infection occurred and he was unable to explain the variation. Chupp[6] failed to secure effective control in the field with calcium hydrate in the early part of the season, although late-season plantings on the same soil were much less severely infected. Wellman secured complete inhibition of infection in the greenhouse with a 2-ton application of calcium hydrate, but this treatment did not preclude root infection in the field, although it did give very good control. In an examination of the numerous reports of liming experiments with clubroot, one is impressed most by the lack of consistent success in disease control in the field.

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FIELD EXPERIMENTS

The field experiments discussed herein were conducted on two badly infested fields. One of these was located near Somers, Kenosha County, Wis., and will be referred to hereafter as the Miller plot. The other was located near Franksville, Wis., and is designated as the Franksville plot. The Miller plot was nearly level except for a low swale which passed through it. The soil was a well-drained Carrington silty clay loam, quite uniform in consistency. The Franksville plot had a slight irregular slope, and the soil was a Clyde silty clay loam. The commercial cabbage crop of 1930 on this area was quite generally infected, but the infection was noticeably least severe on the lower portion of the slope.

EXPERIMENTS ON THE MILLER PLOT

1930 TRIALS

A heavy application of manure was made before plowing. After the land was prepared, 3–10–10 fertilizer was disked in thoroughly at the rate of 1,000 pounds per acre. The liming materials were applied and disked into the soil on June 9 and cabbage (*Brassica oleracea capitata* L.) plants were set on June 20. Two types of hydrate were used. One, which will be referred to as calcium hydrate, had been prepared from limerock consisting of about 95 percent calcium carbonate. The other, dolomitic hydrate, was prepared from dolomitic limestone which contained approximately equal amounts of calcium carbonate and magnesium carbonate.

At the time of liming, the soil was relatively dry and it remained so until transplanting. For 2 weeks after transplanting there was no rain, and the only addition of water during this period was that applied around the plants when they were set. Thus the conditions following transplanting were, according to Monteith,[7] those least favorable for infection, although the "watering-in" of the plants would, according to Wellman[8], be sufficient to favor a certain amount of infection.

The reaction of the soil before lime was applied was pH 5.5, and at the end of the season in the untreated area it was pH 5.8. In the 2-ton calcium hydrate treatment the reaction of the soil changed from pH 5.5 to 7.1, and in the 4-ton treatment to 7.4. In the case of dolomitic hydrate the pH of the soil was somewhat higher, shifting from 5.5 to 7.2 in the 2-ton treatment and to 7.6 in the 4-ton treatment.

General infection became evident by the flagging of the plants during the middle of the day about 8 weeks after they were transplanted. This distinguishing symptom of clubroot infection was apparent over most of the plot. At this time there appeared to be little difference between the treated and untreated areas. It is worthy of note, however, that the cabbage on the area treated with dolomitic hydrate, 4 tons per acre, produced somewhat larger plants than did those on the areas receiving the other treatments.

After the yield data were taken, a large number of plants in each of the treated and untreated areas were pulled and the roots examined to determine the amount and severity of the disease. All the roots examined were more or less clubbed. There was no measurable difference in the extent of infection in the treated and the untreated areas except that in the former the clubs were usually intact, whereas in the latter they were usually completely decayed, indicating that infection has taken place earlier.

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Less than 2 percent of the plants in the treated areas produced marketable heads. The amount of practical control was negligible, and no measurable differences in yield between the four treated areas and the untreated area were secured except on the low area in one portion of the field. In this area, which was favored with a higher level of soil moisture during the growing season, some evidence of the action of the lime as a disease inhibitor was observed. The plants made much better growth as the season progressed, and when the root systems were examined at the end of the growing period a large percentage of plants from the limed portion showed complete inhibition of infection, while in the unlimed portion infection was general.

It is evident from the results of this season that even though calcium and dolomitic hydrate were applied in amounts sufficient to change the reaction of the soil to well above pH 7.0, the amount of infection in most of the plot was not materially affected. This suggested that seasonal or soil conditions influenced the effectiveness of the hydrate in some way not indicated by the pH reading.

1931 TRIALS

In 1931 portions of the same treated and untreated areas of the Miller plot were replanted without further application of liming materials. Another portion of the same field was laid out for further trials. To this various amounts of calcium hydrate and Agstone (finely ground dolomitic limestone) were applied in the autumn of 1930. The field was planted to cabbage on June 1, 1931. Rainfall was again below normal during the growing season.

TABLE 1.—*Effect of various soil treatments upon soil reaction and yield of cabbage on a clubroot-infested soil; Miller plot, 1931*

Material applied	Rate of application per acre	Date of application, 1930	Soil reaction			Yield, September 1931		
			August 1930	June 1931	September 1931	Plants	Marketable heads	Weight of heads per acre
	Tons		*pH*	*pH*	*pH*	*Number*	*Percent*	*Tons*
None	0		5.5	5.8	5.8	2,110	1	0.34
Dolomitic hydrate	2	June 9	7.2	7.1	7.0	246	6	.25
	4	do	7.6	7.4	7.4	243	11	.34
Calcium hydrate	2	do	7.0	7.0	6.8	249	4	.12
	4	do	7.4	7.2	7.2	210	5	.15
	1	Oct. 22	5.5	7.2	7.0	961	17	1.42
	2	do	5.5	7.3	7.1	968	23	2.08
	1	do	5.5	7.1	7.1	971	28	1.98
	2	do	5.5	7.4	7.2	965	26	2.98
Agstone	4	do	5.5	7.4	7.2	462	3	.17
	8	do	5.5	7.8	7.6	471	4	.24

In table 1 is shown the reaction of the soil in the various plots in August 1930, at the time of transplanting in June 1931 and at the end of the season in September 1931. At the time the plants were set the soil in all treated plots was neutral or above. In fact there was then little difference in soil reaction between the plots that had been treated with Agstone and those that had been treated with hydrate in October 1930, or between the plots treated with hydrate in June 1930 and those treated with hydrate in October 1930. In spite of this there was no appreciable reduction in clubroot infection in the hydrate-treated plots of June 1930, nor in the Agstone-treated plots of October 1930. While infection was general in the hydrate plots of October 1930, there were distinct benefits from these treatments, as is shown by the percentage of marketable heads produced. Even in these cases, however, a commercially profitable degree of control was not attained.

EXPERIMENTS ON THE FRANKSVILLE PLOT

1931 Trials

The Franksville plot was plowed in the autumn of 1930, and to certain portions of it 1½ and 2½ tons of calcium hydrate per acre and 4 and 8 tons of Agstone per acre were applied on October 22, 1930. Further treatments of calcium hydrate were applied to other portions at the rate of 1½ and 2½ tons per acre the following spring, one series of treatments being made on April 25, as early as seasonal conditions permitted, and the other on June 2, shortly before the entire plot was planted on June 5.

Table 2.—*Effect of various soil treatments upon soil reaction and yield of cabbage on a clubroot-infested soil; Franksville plot, 1931*

Material applied	Rate of application per acre	Date of application	Soil reaction		Yield, September 1931	
			September 1930	September 1931	Plants	Marketable heads
	Tons		*pH*	*pH*	*Number*	*Percent*
None	0		6.2	6.3	2,078	2
Agstone	4	Oct. 22, 1930	6.2	7.0	864	3
	8	do	6.2	7.4	870	5
Calcium hydrate	1½	do	6.2	7.0	2,074	25
	2½	do	6.2	7.2	2,170	34
	1½	Apr. 25, 1931	6.2	7.0	571	27
	2½	do	6.2	7.2	507	37
	1½	June 2, 1931	6.2	7.1	574	30
	2½	do	6.2	7.2	572	41

In table 2 the effect of the various treatments upon soil reaction and upon yield of marketable heads is given. All treatments changed the reaction to neutral or above. The heavier Agstone treatment gave the highest pH reading, but there was little effect upon clubroot as measured by the percentage of plants which produced marketable heads. The calcium hydrate treatments as a whole were much more effective, the heavier of these treatments raising the pH slightly and giving somewhat higher percentages of marketable heads. None of the hydrate treatments gave what might be considered a commercially successful control. Although there was some reduction in the amount of clubbing as compared with that on the untreated area and on the areas treated with Agstone, the roots of most plants were more or less infected.

The results obtained in 1931 on the Miller plot suggested that fall application might be more effective since in this case it was more influential than the treatment of the spring of 1930. Chupp[9] attributed increasing effectiveness of hydrate during the current season to the time required for the hydrate to become active. In the Franksville experiment a direct comparison between fall, early spring, and late spring applications of hydrate is made. No indication is given that a long interval between application and planting enhanced the inhibition of infection. In fact, the last application, made 3 days before planting, gave slightly better results, although the actual differences are negligible.

1932 TRIALS

The Franksville plot was replanted in 1932, with no further additions of lime. The purpose was to determine whether or not the treatments of 1931 would affect clubroot infection the second season after application. The rainfall was somewhat heavier and more evenly distributed than in 1931. As the season progressed it became increasingly evident that the disease was less severe in the lower portions of the field regardless of treatment. In the final collection of data the percentage of infection and marketable heads was estimated in high and low portions of the same treated area where such an area traversed both levels. The data thus secured are given in table 3. In general the amount of infection was slight in the low portions of the plot and severe in the high portions, irrespective of treatment, and there was no direct correlation between soil reaction and inhibition of clubroot.

TABLE 3.—*Effect of various soil treatments upon soil reaction, yield, and clubroot infection, Franksville plot, 1932*

Material applied	Rate of application per acre	Date of application	Elevation	Soil reaction, September 1932	Marketable heads	Clubroot infection	
						Severe	Slight
	Tons			*pH*	*Percent*	*Percent*	*Percent*
None	0		High	6.9	0	100	0
	0		do	6.9	1	98	0
	0		Low	7.0	57	2	1
	0		High	6.9	1	98	0
	0		Low	7.2	68	2	4
Agstone	4	Oct. 22, 1930	High	6.3	1	98	0
	8	do	do	6.4	1	98	0
Calcium hydrate	1½	do	do	7.0	1	98	0
	1½	do	Low	7.3	49	16	8
	1½	Apr. 25, 1931	High	6.8	14	80	4
	1½	June 2, 1931	do	7.0	12	92	4
	2½	Oct. 22, 1930	do	7.0	1	98	0
	2½	do	Low	7.3	44	4	4
	2½	Apr. 25, 1931	do	7.2	57	4	8
	2½	June 2, 1931	do	7.2	52	0	0

The results of the field trials over a period of 3 years indicated that seasonal and soil factors influence greatly the action of liming materials as clubroot inhibitors. The remainder of this paper is concerned with the study of certain environmental factors in the greenhouse.

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[9] CHUPP, C. See footnote 3.

GREENHOUSE EXPERIMENTS

In pot experiments in the greenhouse, Wellman [10] found that as the chemicals were added to the soil in increasing amounts, infection was inhibited by $Ca(OH)_2$ when the pH reached 7.3, by $CaCO_3$ when it reached 7.9, but when K_2CO_3 was applied until the pH reached 8.1 infection still occurred. This experiment was repeated by the writers and certain other chemicals were included. Untreated soil from the Miller field was used. The experiment was run in three replications in 2-gallon earthenware crocks. The soil moisture was held at 75 to 80 percent of the water-holding capacity. The results are given in table 4.

TABLE 4.—*Effect of application of various chemicals to clubroot-infested soil upon soil reaction and disease development in pots in the greenhouse*

Chemical applied	Rate of application per acre	Series 1			Series 2			Series 3		
		Soil reaction	Healthy plants	Diseased plants	Soil reaction	Healthy plants	Diseased plants	Soil reaction	Healthy plants	Diseased plants
	Tons	*pH*	*Number*	*Number*	*pH*	*Number*	*Number*	*pH*	*Number*	*Number*
Commercial Agstone	1	5.7	0	20	5.8	0	20	5.7	0	20
	2	6.9	3	17	7.0	5	15	6.9	4	16
	4	7.2	20	0	7.2	20	0	7.3	20	0
	8	7.6	20	0	7.5	20	0	7.7	20	0
$CaCO_3$	1	5.5	0	20	5.6	0	20	5.9	0	20
	2	6.9	13	7	7.0	8	12	7.0	9	11
	3	7.0	20	0	7.3	20	0	7.2	20	0
$MgCO_3$	1	6.7	4	16	6.9	6	14	6.9	8	12
	2	7.1	18	2	7.3	17	3	7.2	20	0
	3	7.3	20	0	7.3	20	0	7.2	20	0
Commercial calcium hydrate	1	6.9	3	17	7.1	4	16	7.0	6	14
	2	7.2	20	0	7.4	20	0	7.3	20	0
	3	7.4	20	0	7.4	20	0	7.6	20	0
$Ca(OH)_2$	1	6.8	2	18	7.1	4	16	7.0	5	15
	2	7.2	20	0	7.3	20	0	7.3	20	0
	3	7.6	20	0	7.4	20	0	7.3	20	0
CaO	1	7.1	20	0	7.1	20	0	7.2	20	0
	2	7.3	20	0	7.3	20	0	7.3	20	0
	3	7.8	20	0	7.4	20	0	7.4	20	0
Na_2CO_3	1	5.6	0	20	5.8	0	20	5.8	0	20
	2	5.9	0	20	6.1	0	20	6.1	0	20
	3	6.1	0	20	6.4	0	20	6.3	0	20
K_2CO_3	1	5.7	0	20	5.7	0	20	5.9	0	20
	2	5.9	0	20	5.8	0	20	6.1	0	20
	3	6.1	0	20	6.0	0	20	6.3	0	20
Na_2SO_4	1	5.8	0	20	5.6	0	20	5.6	0	20
	2	6.1	0	20	6.0	0	20	5.8	0	20
	3	6.4	0	20	6.1	0	20	6.1	0	20
Untreated soil	0	5.7	0	40	5.6	0	40	5.6	0	40

Na_2CO_3, K_2CO_3, and Na_2SO_4, in the amounts used, raised the pH only slightly and did not affect clubroot infection. Commercial Agstone, finely ground dolomitic limestone, distinctly inhibited infection when the soil reaction reached pH 6.9 and completely prevented infection at pH 7.2 and 7.6. $CaCO_3$ gave similar results, while $MgCO_3$ was not completely effective until the soil reaction reached pH 7.3. These results differ from those of Wellman, who did not secure complete inhibition with $CaCO_3$ until the pH reached 7.9. $Ca(OH)_2$ produced little effect at pH 6.8 and 6.9 but was completely effective at 7.2 and above, while CaO prevented infection at 7.1.

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[10] WELLMAN, F. L., See footnote 2.

It may be seen in this experiment that in the greenhouse those chemicals that were applied in amounts sufficient to raise the soil reaction slightly above neutral were in general very effective in preventing infection. This is in direct contrast to the results of field experiments described above in which the application of some of the same materials in quantities which raised the pH to 7.0 to 7.8 usually failed to prevent an abundant development of disease. As was pointed out earlier, in the Miller plot in 1930 and in the Franksville plot in 1932, calcium hydrate was effective in low portions of the fields where the soil moisture was relatively higher and conditions for plant growth were more favorable. In the greenhouse experiments a reasonably constant and favorable soil moisture was maintained and here also the liming materials were effective.

In the autumn of 1930 soil was secured from the untreated area and from each of the areas which had received 2- and 4-ton applications of calcium hydrate in the 1930 trials on the Miller plot. The reaction of the untreated soil was pH 5.8, that of the soil from the 2-ton area was pH 7.1, and that from the 4-ton area was pH 7.4. The three lots of soil were placed in 2-gallon earthenware jars and kept at 70 to 80 percent of the water-holding capacity. Twenty plants were grown in each sample for 1 month and the experiment was replicated at three different times. Heavy infection with clubroot was secured throughout the series in every plant on the untreated soil. No infection whatever occurred in the soil from the treated areas. In the autumn of 1931 soil was collected from the areas of the Miller plot which had received 4-ton and 8-ton applications of Agstone in October 1930. It may be recalled that in the 1931 season there had been no evidence of disease inhibition in these areas (table 1). At the time of collection the soil from the 4-ton Agstone area had a reaction of pH 7.4, and that from the 8-ton area had a reaction of pH 7.8. This soil was set up as described in the last experiment, and untreated soil was again used as a control. Forty plants were grown in each sample of soil for approximately 4 weeks. At the end of that time all plants in the untreated soil were severely diseased, none was diseased in the soil receiving the 8-ton treatment, while in that receiving the 4-ton treatment about 50 percent of the plants were infected. The experiment was repeated and the outcome was similar. These results gave conclusive evidence that the conditions surrounding the experiment in the greenhouse permitted complete control in the soil treated with 2-ton and 4-ton applications of calcium hydrate and an 8-ton application of Agstone, while conditions in the field had allowed heavy infection in the same soils. These results conform with those of other workers, who have secured consistent and convincing evidence of the effectiveness of lime in greenhouse tests while in the field various degrees of inconsistency have prevailed.

A study of this soil was continued by taking samples from the calcium-hydrate-treated areas over a period of about 2 years, and conducting greenhouse trials in the manner described above. The results are given in table 5. It is to be noted that as the interval after treatment increased the reaction of the treated soil gradually reverted to neutral and below. As the soil became more acid the percentage of infected plants gradually increased.

TABLE 5.—*Clubroot infection in field-treated soil when removed to the greenhouse at various intervals after treatment*

Treatment	Greenhouse tests with soil collected after—											
	2 months			12 months			17 months			23 months		
	pH	Plants used	Plants infected	pH	Plants used	Plants infected	pH	Plants used	Plants infected	pH	Plants used	Plants infected
		Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*		*Number*	*Percent*
Calcium hydrate, 2 tons per acre	7.1	70	0	7.0	70	18	6.8	70	68	6.4	70	90
Calcium hydrate, 4 tons per acre	7.4	70	0	7.3	70	0	7.0	70	0	6.7	70	40
Dolomitic hydrate, 2 tons per acre	7.2	70	0	7.1	70	12	6.9	70	56	6.4	70	70
Dolomitic hydrate, 4 tons per acre	7.6	70	0	7.4	70	0	7.1	70	0	6.9	70	30
Untreated	5.8	70	100	5.8	70	100	5.6	70	100	5.6	70	100

In the 1930 and 1931 field trials the soil moisture was relatively low for a major portion of the time, while in the greenhouse experiments it was kept consistently high. In order to ascertain whether soil moisture was a determining factor in the effectiveness of lime, soil from both 2-ton treatments with calcium and dolomitic hydrate in the 1930 Miller plot was set up at fairly constant soil-moisture levels of about 40 to 50 percent, 55 to 65 percent, and 70 to 80 percent of the water-holding capacity. Plants were transplanted to the crocks and the moisture levels maintained for 5 weeks. At the end of that time the plants in the untreated soil were uniformly infected at all moisture levels. In treated soils there was complete inhibition of infection at all moisture levels. The experiment was extended to the 8-ton Agstone-treated soil from the 1931 Miller plot. This soil was held at moisture levels of 40 to 50, 50 to 60, 60 to 70, 70 to 80, and 80 to 90 percent of the water-holding capacity. After 4 weeks all plants on the untreated soil held at 70 to 80 percent of the water-holding capacity were diseased. There were no diseased plants in the Agstone-treated soil at any of the moisture levels. The entire experiment was repeated with identical results.

Although the effectiveness of the two types of hydrate and Agstone as inhibitors of infection was uniform over a range of relatively constant soil-moisture levels, the conditions which prevailed still did not simulate those in the field, where there is usually a fluctuation of the moisture content of the soil. The next experiment was set up to observe the effect on infection of fluctuation in soil moisture.

Untreated soil and soil from the 2-ton treatment with calcium hydrate in the 1930 Miller plot were used in the winter of 1930–31. Two earthenware crocks were filled with the calcium hydrate field-treated soil and two with untreated soil. Ten plants were set in each crock and the moisture content of the soil was held at 70 to 80 percent of the water-holding capacity. Another lot of the treated soil was allowed to dry out until the moisture content was below 16 percent of the water-holding capacity. Four flats 20 inches long, 14 inches wide, and 12 inches deep were filled to a depth of 9 inches. Six plants were set in each flat, each plant being watered in with 30 cc of water. After 5 days 50 cc of water was added to each flat by spraying it uniformly over the surface. After another 5 days 100 cc was added in the same manner and this procedure was repeated every 5 days during the remainder of the 6 weeks' duration of the experiment. Thus, in the earthenware crocks the soil moisture was quite constant and relatively high throughout the experiment. In the flats, however, the conditions resembled more closely those in the field trials, where plants were watered into relatively dry soil. The high moisture content of the soil around such transplants, besides being sufficient for infection, stimulated emergence of new secondary roots, which soon grew into the soil. The moisture content of this soil varied considerably from day to day and at different distances from the surface.

After 6 weeks the plants from all crocks and flats were removed and the experiment was repeated. The results of both series are given in table 6. In the crocks in which the moisture content was kept reasonably constant the results of previous experiments were duplicated. Every plant in the untreated soil was infected, but there was no infection in the treated soil. On the other hand, in the flats in which the moisture around the plants was relatively high at the time of transplanting, but was allowed to fluctuate in the whole bulk of the soil, every plant was infected. It is true that the average soil moisture in the flats was lower than in the same soil in the crocks, but infection cannot be attributed to low soil moisture alone since in previous experiments constant low soil moisture failed to favor infection. It appears that the fluctuation of soil moisture at relatively lower levels provided the proper conditions for infection in spite of the fact that calcium hydrate had been incorporated in the soil. Under these conditions the organism infected the plants readily as it had done earlier in the 1930 Miller plot.

TABLE 6.—*Clubroot infection in calcium-hydrate-treated and in untreated soil at constant and fluctuating soil moisture*

Treatment	Condition of soil moisture	Series 1		Series 2	
		Plants used	Plants infected	Plants used	Plants infected
		Number	*Percent*	*Number*	*Percent*
Untreated	Held at 70 to 80 percent of the water-holding capacity in crocks.	20	100	20	100
Calcium hydrate, 2 tons per acre	do	20	0	20	0
Do.	Started at 16 percent of the water-holding capacity, and allowed to fluctuate in flats	24	100	24	100

Similar lots of treated and untreated soil were next set up in 2-gallon earthenware crocks at 70 to 80 percent of the water-holding capacity. The untreated soil and part of the crocks containing treated soil were held at constant high soil moisture by daily weighing and addition of water. The remaining crocks of treated soil were allowed to dry out gradually until the soil moisture approached 40 percent of the water-holding capacity. They were then brought up to the original moisture content by the addition of water. This process was repeated twice during an interval of 4 weeks. When the plants were removed and examined all those in the untreated soil were diseased. In the treated soil no infection occurred where the moisture was held constant nor where the moisture level fell and rose periodically. Thus this type of fluctuation had the opposite effect from that in which fluctuation at low soil moisture occurred.

Another experiment was conducted to determine the effect of aeration of treated soil upon infection. Two-ton calcium-hydrate-treated soil was placed in 2-gallon earthenware crocks and a perforated soft lead tube was incorporated in the soil. By this means a continuous flow of air was forced through the soil. An equal number of nonaerated crocks were used. During the course of the experiment the soil moisture fluctuated from 65 to 80 percent of the water-holding capacity. After 30 days the plants were removed and the roots examined. The results of two series of experiments, given in table 7, show that the introduction of air into the treated soil resulted in 35 to 40 percent infection, while in nonaerated treated soil only 1 plant in 40 became infected. In untreated nonaerated soil all plants were severely diseased.

TABLE 7.—*Effect of aeration of calcium-hydrate-treated soil upon clubroot infection*

Soil treatment	Aeration	Series 1		Series 2	
		Plants used	Plants infected	Plants used	Plants infected
		Number	*Percent*	*Number*	*Percent*
Calcium hydrate, 2 tons per acre	Aerated	20	35	20	40
	Nonaerated	20	5	20	0
Untreated	do	20	100	20	100

DISCUSSION

Under the climatic conditions of 1930 to 1932, inclusive, hydrated lime did not uniformly inhibit clubroot infection in the field, although the soil reaction was in many cases changed to pH 7.0 or higher. However, when the same soil was removed to the greenhouse and plants were grown in it, the treatment was completely effective in preventing infection. Under the latter condition, where frequent watering was carried out, calcium hydrate, calcium carbonate, calcium oxide, and magnesium carbonate all reduced infection perceptibly when added in amounts sufficient to raise the pH to about 7.0, and usually inhibited infection completely at pH 7.2 or above. High or low relatively constant soil moisture did not change the degree of inhibition. Fluctuation of soil moisture and forced aeration, however, did permit varying degrees of infection in soil. It appears that fluctuation in soil moisture is likely to permit infection in slightly alkaline field soils.

While this study does not explain the fundamental bases of the effectiveness of lime as a clubroot inhibitor under various conditions, the results do show significant differences between greenhouse and field trials. It is obvious that greenhouse pot tests are not a true index of what may be expected in the field. If one were to consider the greenhouse results given in table 4 alone there is ground for concluding that a distinct correlation exists between soil reaction and infection by the clubroot organism. Why this does not hold in the field is not explained, but it is clear that when the soil moisture fluctuates at relatively low levels abundant infection may occur at soil-reaction values which give no infection under the conditions of the experiments recorded in table 4.

It is suggested in this connection that the soil-reaction determination as ordinarily made is not necessarily a true index of the actual pH value of the soil closely adjacent to the roots. Thom and Humfeld [11] studied the reaction of soil particles adherent to fibrous root from a number of crop plants grown in a range of acid and alkaline soils. The soil in this zone, compared with the soil mass as a whole, was less acid when an acid soil was used, and less alkaline when an alkaline soil was used. It is possible that in the slightly alkaline treated soil employed in this study, the reaction in the immediate vicinity of the roots was sufficiently acid, under field conditions, to permit abundant infection. It is evident that treatment of soil sufficient to inhibit infection in the greenhouse in no sense eliminates the organism. When the reaction of such a soil naturally reverts to below pH 7.0 abundant infection occurs even when favorable soil moisture is maintained (table 5).

These results are offered only as they apply to the two soil types used. It is quite possible that heavier soils, higher in water-holding capacity, would yield entirely different results.

SUMMARY

Field treatments of Carrington silty clay loam and Clyde silty clay loam with calcium hydrate and calcium magnesium carbonate which raised the reaction to pH 7.1 and above did not generally inhibit clubroot development.

In treated soils, removed to the greenhouse, cabbage plants remained free from infection while in untreated soil from the same field there was abundant disease development.

Under greenhouse conditions infection was inhibited in treated soil at high, intermediate, and low relatively constant moisture levels.

Calcium hydrate, calcium carbonate, calcium oxide, and magnesium carbonate all reduced infection perceptibly in well-watered soil when added in sufficient amounts to raise the reaction of an acid soil to about pH 7.0, and usually inhibited infection completely at pH 7.2 or above.

Fluctuation of soil moisture at a relatively low soil-moisture level and forced aeration of the soil permitted varying degrees of infection in treated soils.

It is suggested that low fluctuating soil moisture is an influential factor in limiting the effectiveness of lime as a clubroot inhibitor in the field.

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[11] THOM, C., and HUMFELD, H. NOTES ON THE ASSOCIATION OF MICROORGANISMS AND ROOTS. Soil Sci. 32: 29-36. 1932.

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LIFE HISTORY OF THE CROWN-GALL ORGANISM IN RELATION TO ITS PATHOGENESIS ON THE RED RASPBERRY[1]

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INTRODUCTION

Crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. (*Bacterium tumefaciens* Smith and Town.), is a serious and wide-spread disease of cane fruits. Though much attention has been given to various other phases of the crown-gall problem, comparatively little study has been made of it on cane fruits, and the life history of the causal organism in relation to pathogenesis has been but imperfectly understood. The present investigation was undertaken in an effort to define important points in the life history of the causal organism in relation to its pathogenesis on the red raspberry (*Rubus strigosus* Michx.) in the hope that such knowledge might contribute toward the development of control measures.

One strain of *Phytomonas tumefaciens* was used in most of the experiments reported herein. This strain was originally isolated by the writer from a crown gall on Wealthy apple. It has been described as strain 2018 in studies recently reported by Riker et al. (*27*, *28*),[3] and was repeatedly demonstrated throughout the entire course of these investigations to be highly pathogenic on tomato, *Sedum*, tobacco, apple, *Bryophyllum*, sugar beet, and the underground parts of both red and black raspberry varieties. The reactions on several common laboratory media and host plants, induced by 18 strains of the crown-gall organism isolated by the writer from as many collections of crown gall on the roots of red raspberry from Wisconsin, Indiana, and Michigan, have not differed from those induced by strain 2018.

EXIT OF THE CROWN-GALL ORGANISM FROM THE HOST

It has frequently been considered that the crown-gall organism is released from the galls only at the time of their disintegration. From the recent experimental work discussed herein it has been found, however, that under favorable moisture conditions crown-gall bacteria are continuously passed off from the surface of living crown galls.

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[2] The writer takes pleasure in acknowledging the advice and encouragement given him during the course of this work by the members of the Departments of Plant Pathology and of Economic Entomology of he University of Wisconsin. He also wishes to express his indetedness to Prof. A. A. Granovsky, formerly of the Department of Economic Entomology of the University of Wisconsin, for assistance in preparing he illustrations.

[3] Reference is made by number (italic) to Literature Cited, p. 785

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That the soil in which diseased plants are grown contains the crown-gall organism has been suggested by several investigators, who have reported that susceptible plants grown in such soil become diseased (*1, 2, 13, 17, 21, 25, 34, 35, 38*). Patel (*22*) has reported successful isolation of the crown-gall organism from the soil of nursery fields that had produced crops affected by the disease. It has commonly been assumed that the bacteria in the galls are liberated when the galls decay (*5, 6, 17, 18*). The limited experimental evidence does not sustain this widely held assumption. Riker and Keitt (*29*) reported that "from two dozen recorded attempts at isolation from decaying crown-gall tissue, no crown-gall bacteria were found." The present writer has likewise been unsuccessful with either potato-dextrose or bile-agar media (*22*) in many isolation trials from decaying or decayed crown-gall tissue. Robinson and Walkden (*31*) reported that they had observed great numbers of crown-gall bacteria on the surface of living crown galls and that prolonged exposure to running water reduced the bacterial population on the surface by about 200 to 1.

Each of two active crown galls free from decay was successively immersed for different periods in a series of equal volumes of sterile water (table 1). Upon transfer of the gall from one quantity of water to the next in the series, a bacterial plate count was made of the water in which the gall had just been immersed. The results (table 1) are from (1) a crown gall induced on the underground portions of a tomato stem by inoculation about 30 days prior to the time of the experiment, and (2) a crown gall induced by inoculation on the underground portion of the scion of a vigorously growing Wealthy apple graft about 10 weeks prior to the time of the experiment. The bacterial counts made of the successive wash waters show (1) that during the time of the experiment the bacteria were passing from the surface of the gall to the surrounding water, and (2) that the number of bacteria given off to the water during any immersion period was a very small fraction of the total bacterial population of the gall.

TABLE 1.—*Number of crown-gall bacteria found in successive 10 cc volumes of sterile water in which a crown gall had been immersed for the time indicated in the sequence given*

Specimen and treatment	Duration of treatment	Bacteria found in water
Tomato crown gall:	*Minutes*	*Number*
Immersed in water [a]	10	4,050,000
Do	30	4,800,000
Do	10	697,000
Do	10	2,717,000
Do	30	13,470,000
Do. [b]	10	1,867,000,000
Apple crown gall.		
Immersed in water [a]	10	3,267,000
Surface washed in running water [c]	35	
Apple crown gall—contd.	*Minutes*	*Number*
Immersed in water [a]	10	2,120,000
Do	40	49,670,000
Do	10	893,400
Surface washed in running water [c]	70	
Immersed in water [a]	10	264,120
Do	50	19,463,000
Do. [b]	10	550,000,000

[a] Immersed in 10 cc of sterile water in a large test tube.
[b] Macerated and placed in 10 cc of sterile water.
[c] Placed on cheesecloth in a stream of water falling from a faucet.

Aerial portions of the stems of a number of succulent tomato plants were sealed in small cylindrical glass chambers within which the bacterial flora could be controlled. These cylinders were 1¼ inches in diameter by 3 inches long, with a T side-arm extension of glass tubing one half inch in diameter and three fourths of an inch long. They were slipped down over the leaves of young tomato plants and held in place by clamps supported on ring stands. The open ends of the cylinders were closed by thin sections of a one-hole rubber stopper of suitable diameter. Adhesive tape was bound over the outside of the stoppers and the ends of the cylinders. The ends were then sealed with a mixture of vaseline and paraffin, which was poured in a liquid state through the side arm and around the stem at the bottom of the cylinder. After the mixture had hardened, a thin layer of liquid vaseline was placed on top of the paraffin-vaseline mixture. The top end of the cylinder was sealed by holding the potted plant with the attached cylinder upside down. All motion of the stem within the cylinder that might break the vaseline seal in subsequent manipulations was prevented by binding the pot in which the plant was growing to the base of the ring stand supporting the cylinder (fig. 1).

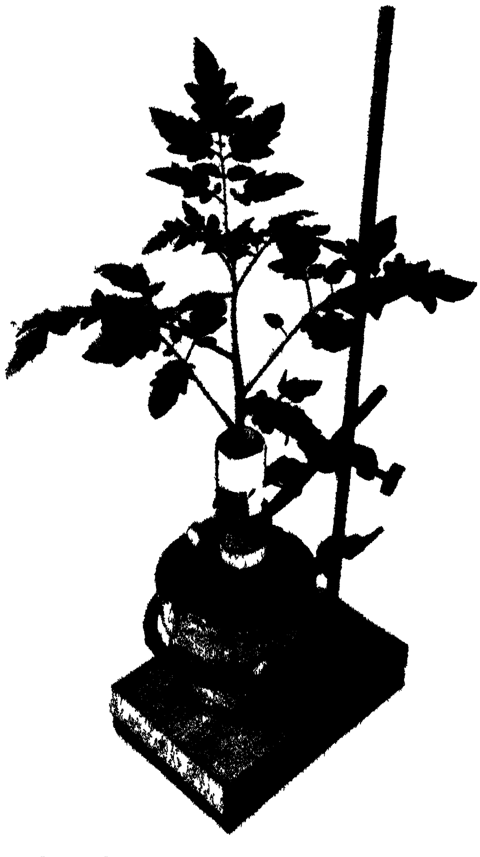

FIGURE 1.—Glass cylinder sealed over a section of tomato stem. In this manner sections of tomato stem were held for short intervals of time under aseptic conditions.

The surfaces of those portions of the stems which were sealed in the cylinders were then disinfected, inoculated, and again disinfected. Disinfection was accomplished by filling the cylinders for 10 minutes with silver nitrate solution (1:1,000), introduced, as were all materials subsequently employed, through the side arm of the cylinder, the free end of which was closed by a removable rubber stopper. After the silver nitrate solution had been removed, the cylinders were washed with three successive changes of sterile water, each of which was held in the chamber for 10 minutes.

A plate count was made of a fourth change of sterile water as a check on the effectiveness of the disinfection. One day after disinfection the sterile surfaces of the stems within the cylinders were inoculated by thrusting into each stem a needle that had been smeared with a crown-gall culture. As protection against contamination, cheesecloth moistened with silver nitrate solution was held over the cylinder during this and other manipulations. Two days after inoculation the stem surfaces within the cylinders were again disinfected, and a plate count similar to that just described was made as a check on the effectiveness of the disinfection.

Two months later an estimate was made of the number of crown-gall bacteria on the surfaces of the stems and large galls induced within the chambers by inoculation. This estimate was obtained by plate counts made of sterile water with which the chamber had been filled and in which the gall had been immersed for 10 minutes. The results from three cylinders in which galls had been successfully induced by the method just described showed that immediately after the first and the second disinfections no crown-gall bacteria were present on the surface of the stem. Moreover, no bacterial or fungal colonies developed in these plates. But 2 months later the plate counts showed 1,000,000, 4,000,000, and 24,000,000 crown-gall bacteria present on the surface of the stems and galls enclosed, respectively, by the three cylinders.

Before disinfection of the gall surfaces, immediately after disinfection, and at various intervals of time thereafter, estimates were made of the bacterial population on the surfaces of three other tomato crown galls held under aseptic conditions within the glass cylinders just described. Before disinfection an average of 3,750,000 living crown-gall bacteria were found. Thirty minutes after disinfection there was none; 4 hours later there were 4,000; 36 hours after disinfection, 13,500; and 5 days after disinfection, 4,400,000.

LONGEVITY AND OVERWINTERING IN SOIL

Observations over a number of years have led to the belief that the crown-gall organism may live for some time in soil in which diseased plants have grown (*1*, *2*, *13*, *21*, *34*, *35*). Experimental evidence reported herein demonstrates that the crown-gall organism overwinters in the soil under ordinary field conditions and that it may live for more than a year in unsterilized soils held free from the growth of seed plants.

Experimental evidence as to the overwintering and longevity of the crown-gall organism has been reported by several writers (*3*, *19*, *23*, *24*). Muncie (*19*) states that tomato plants transplanted to inoculated soil became infected until 154 days after the soil had been inoculated. Patel (*23*) reported that he was able to recover the organism in a pathogenic state from inoculated unsterilized soils in which it had overwintered under field conditions at Ames, Iowa. From a series of isolation trials made from various types of inoculated unsterilized soils held moist under laboratory conditions, he concluded that the organism "may live longer in sandy soils than in clays." He was able successfully to isolate and demonstrate the pathogenicity of the organism 669 days after inoculation in unsterilized loam. On the basis of isolations from the soils of a number of nursery fields, Patel reported that "the pathogenic form of *Ps. tumefaciens* is localized in the field, * * * in close proximity to true galls", and that "organisms resembling

Ps. tumefaciens are not generally found in soils from which crops susceptible to this pathogene are absent."

To determine whether the organism overwinters under field conditions at Madison, Wis., two plots of soil 3 feet square were inoculated September 23, 1926, and isolations were attempted in November and again in May of the following spring. Two diverse soil types were chosen, one a neutral peat and the other a Clyde silt loam. Inoculation to a depth of 2 feet was accomplished by removing the top layer of soil and treating the underlying layers first with a heavy suspension of *Phytomonas tumefaciens*. This was prepared by diluting, at the rate of 1 culture per 6 quarts of water, 6-day mass cultures grown on agar slopes in flat 11-ounce bottles. The suspension was mixed at once with the soil at the rate of 1 pint per cubic foot of soil. Soil samples for isolation were taken from 4 to 7 inches below the surface of the soil in each plot. Soil dilutions of 1:1,000, 1:10,000 and 1:100,000 were used to pour agar plates with Patel's bile medium (*22*). Five plates were poured for each dilution and were incubated 6 days at 28° C. They were then examined for crown-gall-like colonies, a number of which were selected, and with each colony from 2 to 10 inoculations were made on tomato stems. The ability of these bacteria to induce galls when inoculated into susceptible tomato stems was the criterion used in identifying the crown-gall organism. The results, recorded in table 2, show that the organism overwintered in the soil of these two fallow field plots.

TABLE 2.—*Overwintering of Phytomonas tumefaciens in 2 types of soil under field conditions at Madison, Wis., 1926-27* [a]

Date of isolation	*P. tumefaciens* from neutral peat soil			*P tumefaciens* from Clyde silt-loam soil		
	Colonies tested	Total inoculations	Inoculations positive	Colonies tested	Total inoculations	Inoculations positive
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Nov. 12, 1926	20	40	85	20	40	95
May 21, 1927	17	68	68	34	136	15

[a] The soils were inoculated on Sept 23, 1926.

To gain information as to the length of time the organism might exist in the soil in a pathogenic state, isolations were attempted periodically from two samples of unsterilized inoculated soil. The types used were the neutral-peat and silt-loam soils referred to above. They were held moist and free from the growth of seed plants in open cans in a cellar used for the storage of nursery stock. The isolation technic used and the criterion of successful isolation were the same as in the overwintering studies. The degree of success was measured by the percentages of the inoculations made with these colonies that induced crown galls on tomato stems. From the data recorded in table 3, it is clear that the organism can live for over a year in a pathogenic state in the soil in the absence of seed plants.

When inoculated into tomato stems, the last crown-gall colonies isolated from the silt-loam soil induced but very small galls. Four inoculations were made into each of two tomato stems with the three crown-gall-like colonies isolated December 7, 1926. All the inoculations made with two of these colonies were negative. Seven of the eight inoculations made with the third colony produced uniform galls that were, however, very small as compared with the galls induced by parallel inoculations from a 6-day-old transfer of the stock culture with which the soils had been inoculated. Correspondingly, one of the four colonies isolated March 3, 1927, induced relatively small overgrowths on tomato. Although other crown-gall-like colonies were isolated in these and in subsequent trials, none induced any overgrowth response whatsoever in similar pathogenicity tests. These results suggest that the ability of the organism to induce galls on tomato may have declined after it had lived for a long period in the soil; or the small size of the galls induced by the last colonies isolated may have been due to competition with other organisms contained within these colonies (*7, 27, 43*).

TABLE 3.—*Longevity of Phytomonas tumefaciens in samples of 2 unsterilized soils kept in a storage cellar at Madison, Wis., 1926–27* [a]

Date of isolation	*P. tumefaciens* from neutral peat soil			*P. tumefaciens* from Clyde silt-loam soil		
	Colonies tested	Total inoculations	Inoculations positive	Colonies tested	Total inoculations	Inoculations positive
1926	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
May 14	10	20	100	10	20	100
May 31	10	20	100	10	20	100
June 11	10	20	100	10	20	100
June 24	10	20	100	10	20	100
July 25	10	20	100	10	20	80
Sept 10	10	20	100	10	20	0
Nov. 7	10	20	100	10	20	70
Dec 7	5	15	100	3	24	29
1927						
Mar. 3	10	40	80	4	24	25
July 2	10	40	70	6	36	0
Oct. 10	5	20	0	1	8	0
Nov. 15	5	20	0			

[a] The soils were inoculated on May 1, 1926, with pure cultures and held under approximately constant moisture conditions at 17° C

DISSEMINATION

That crown gall is spread to new areas by the shipment of infected nursery stock has long been known, and careful inspection has often been relied upon to prevent this spread. The writer's experimental evidence discussed herein indicates that rigid inspection of raspberry plants does not prevent the disease from being spread in this way, because incipient stages of infection, which no amount of careful inspection can detect, may be carried by apparently healthy plants.

Selby (*33, pp. 111 112*) reported the disease to have occurred in a number of raspberry plantations in the vicinity of Florence, Ohio, and stated that "* * * all the growers procured the plants from the same source. The plantation from which these plants came was found, on examination, to be diseased in the same manner." Lawrence

(*17*, p. *17*) 10 years later warned Washington growers against using plants from diseased fields, and said:

> * * * even if only those plants are used which do not show signs of the galls the trouble will be quite sure to manifest itself at a later period. The writer has seen this occur in a number of instances.

Bennett (*6*, p. *22*) reported:

> Plants which have been heeled in or which have been held in cold storage and wet down several times, although they may have few visible galls when planted in the field, have been known to become severely diseased often before the end of the first season. Apparently, in such cases, the bacteria are washed from the few galls which may be in the bundles and find their way into wounds where they become established but do not produce visible swellings till after planting.

Investigators who have studied crown gall have usually found it difficult to obtain plants free from the disease. Thus Smith et al. (*39*) could draw no conclusions from certain inoculation experiments on red raspberry because of the infection that appeared on the roots of the uninoculated control plants. They state that "the infection was probably brought along with them from the nursery, because one or two knots were found on the roots of these plants when they were purchased." Colby's experience, as reported by Keilholz (*16*), in conducting experiments on control of crown gall on raspberries by means of germicides has been similar. He states that conflicting results come

> * * * from not knowing whether the plants were clean when planted. An attempt was made to set only clean plants, but it is impossible to detect the crown-gall infection in its incipient stage. If the plant is already infected at planting time, the infection is quite certain to enlarge unless the soil near the plant has been so heavily treated with the germicide that the plant itself is killed.

A test was made by the writer to determine whether apparently healthy red raspberry plants carry incipient stages of infection. Plants of the Latham variety were obtained from a nursery in which the disease was known to be present. These plants were carefully examined for macroscopically visible infections before planting. A number of such plants were found bearing galls 1 mm or more in diameter. These were discarded. Plants selected as not visibly diseased were completely submerged for 20 minutes in a silver nitrate solution (1:1,000) to prevent contamination by crown-gall bacteria that might be present on the surface. They were then set out in beds of soil which had been steamed.

These beds had been heated by the inverted-pan method of high-pressure steaming described by Johnson (*15*). Each bed after being steamed for 30 minutes with a head of 120 pounds pressure, was surrounded by boards as protection against contamination by surface water and left for 1 week before planting. Ten beds thus prepared were planted May 12, 1928, with 600 raspberry plants that had been selected and treated as described.

Twenty-five to fifty raspberry plants were dug and examined at intervals of 2, 3, 11, 12, and 18 months after planting. Of 344 plants harvested from these steamed beds during this period, 51, or 15 percent, were affected with crown gall. An average of 1.7 infections was found on diseased plants harvested within 1 year after planting. The size and condition of the galls found on plants during the first growing season indicated that infection had occurred prior to or soon after the time of planting. Since it seems improbable that crown-gall organisms in the soil were not killed by the steaming or that the soil was reinfected during the first growing season, it appears that the galls which developed on these plants were due to incipient infections which were present on the plants when received from the nursery but which were not visible. This view is supported by further evidence reported in a later section dealing with (1) the protracted incubation period of crown-gall infections under unfavorable growing conditions and (2) the length of time wounds remain potential avenues of infection by *Phytomonas tumefaciens*. On the raspberry, the disease has been reported by Bennett (*6*) to occur through wounds, and by Dodge and Wilcox (*9*) "through wounds made by insects or by mechanical injuries." The Great Britain Ministry of Agriculture and Fisheries (*12*) very succinctly summarizes the literature on this subject by saying:

> It is generally believed that infection can occur only through wounds, for practically all attempts to produce Crown Gall by inoculation of unwounded tissues have given negative results, whilst the frequent occurrence of Crown Galls on parts of plants which have been cut or wounded strongly supports the view that the organism is a wound parasite. The fact that Crown Galls occur on roots and occasionally on seedlings where no evidence of wounding is apparent does not negative this view, for insignificant wounds made by soil insects or by other means would suffice as ports of entry for the organism.

Observations and experiments have been made by the writer, as previously reported in abstract form (*4*), to determine (1) whether injuries on the underground parts of the red raspberry remain subject to infection for relatively long periods, (2) whether in the absence of injuries to the underground parts of the plant crown-gall infection may occur, and (3) whether root-feeding arthropods are chiefly responsible for the injuries through which crown-gall bacteria gain entrance to the tissues of the plant. The details of this work follow

LENGTH OF TIME INJURIES ON HOST REMAIN OPEN TO INFECTION

The pronounced susceptibility of the red raspberry to crown-gall infection might be explained in some measure were it demonstrated that injuries on the underground parts of this plant remain open to crown-gall infection for relatively long periods. This phase of the problem was studied in four series of experiments over a period of 3 years. Plants in various stages of development were studied under both field and greenhouse conditions. In both types of experiment all injuries were made at one time and inoculum was applied at various intervals after wounding. The injuries in all cases were made by scraping through the cambial layer with a scalpel. The plants used were grown in soil that had previously been steamed, and precautions were taken against contamination of the injuries by crown-gall bacteria before the inoculum was applied. In experiments on plants in the field, because of the comparative inaccessibility of the roots, most of the injuries were made on the underground portion of the stem. After the soil around the points of injury had been removed, the inoculum was applied to the injuries by means of a camel's-hair brush. Every precaution was taken not to disturb the tissues at the points of injury when the soil was removed and the inoculum applied. Examination of the wounds was made several months after the inoculum was applied to the last set of injuries in the series. The results of the field studies made in this manner (table 4) indicate that injuries on the underground parts of the red raspberry in some cases may remain open to invasion by the crown-gall organism for 6 weeks.

TABLE 4.—*Length of time during which crown-gall infection was secured through injuries made on stems of red raspberry plants in the opening-bud stage of early spring*[a] *and in the early autumn*[b]

Period between injury and inoculation (days)	Plants injured	Injuries inoculated[c]	Injuries infected at close of season
OPENING-BUD STAGE OF EARLY SPRING			
	Number	*Number*	*Percent*
1	7	28	57.1
2	5	20	70.0
6	6	24	54.2
18	7	28	43.0
Controls	15	60	0
EARLY AUTUMN			
2	21	72	77.8
5	17	66	57.6
9	13	43	16.3
42	[d] 13	44	15.2
Controls	20	60	1.6

[a] The injuries were made on the underground section of the stems of red raspberry plants, just before planting them in steamed soil on May 8, 1928

[b] The injuries were made Sept. 1, 1928, on the underground sections of the stems of red raspberry plants grown in steamed soil

[c] A suspension of crown-gall bacteria was applied to the injuries with a camel's-hair brush at various intervals after the time of injury

[d] These plants were practically in a dormant condition when the inoculum was applied to the injuries The results were recorded at the close of the following season.

ENTRANCE INTO HOST

"Everything we know about crown gall points to wounds as the usual, if not the only way of infection" (*38*). This view, held by Smith in 1920, has been supported by the observations (*10*, *11*, *17*, *19*, *29*, *32*, *41*, *42*) and experiments of all later investigators.[4] Infection has been obtained experimentally on apple (*19*, *20*, *27*, *29*, *36*, *37*), tomato (*19*, *26*), *Ricinus* (*26*, *30*), geranium (*30*), and grape (*17*, *18*) only through wounds.[5]

Under greenhouse conditions similar studies were made on the roots of red raspberry plants grown in root-observation boxes. These plants had been grown for 1 year in the steamed-soil plots previously described and were free from crown-gall infections when set out in the boxes in the spring, since those that carried latent infections from the nursery had been detected and discarded. The soil in the root-observation boxes was steamed at the beginning of the experiment. In August injuries were made on the roots in the manner already described, and at various periods of time thereafter inoculum was applied to them. Several months after the last group of injuries in the series was treated, all the injuries were examined and the presence or absence of the disease was recorded.

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[4] A possible exception is Stapp (*40*), who reported that tumors caused by *Bacterium* (*Phytomonas*) *tumefaciens* occurred at the eyes of potatoes that were planted either in inoculated soil or in ordinary garden soil after being dipped in a suspension of the organism.

[5] Experiments made by various investigators with *Phytomonas rhizogenes* (*28*), often called the apple strain of the crown-gall bacterium, are not included, although recent research (*14*) on this organism shows that it gains entrance to the tissues of its host primarily if not solely through injuries.

Root-observation boxes of a type similar to those described by Dean (*8*) were used in these experiments. The sides of the boxes were made of wooden hinged doors that covered removable glass plates. The wooden doors could be opened readily and through the glass plates the roots in contact with the glass at the surface of the soil could be observed without disturbing them. The glass plates were removed when the injuries and inoculations were made. The use of these boxes made it possible to manipulate and observe the roots without danger of causing further injury by removing the surrounding soil, as must be done in field experiments. By the middle of August the roots in the boxes had grown extensively and a considerable number were in contact with the glass plates at the sides of the boxes.

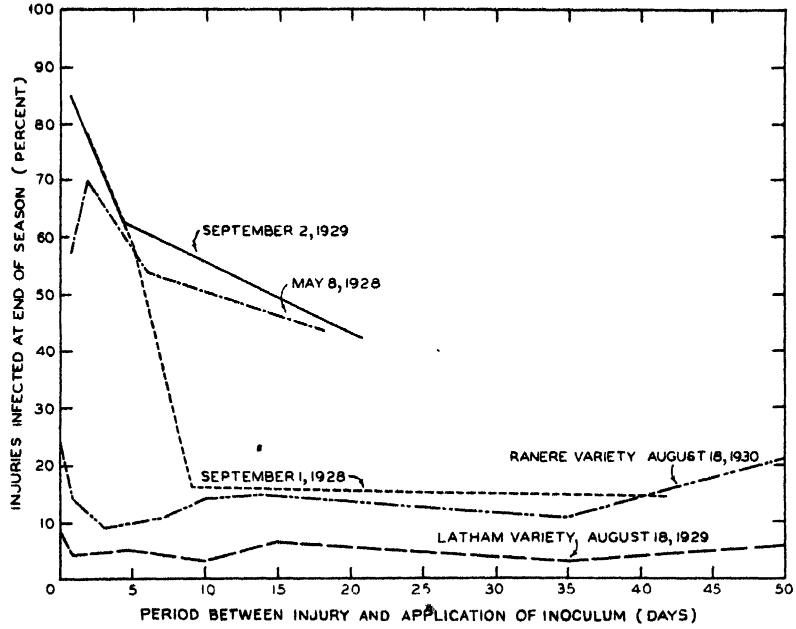

FIGURE 2.—Length of time during which crown-gall infection was secured through wounds on underground parts of red raspberry. The dates given are the dates at which the injuries studied in the experiments were made

Injuries were made with a scalpel, as in the field experiments. The injuries were then marked by means of map tacks in the ends of match sticks that were pushed into the soil so that the head of the map tack was adjacent to the injury at the surface of the soil. The injuries in each group in the series were made on the roots of plants in 1 or 2 boxes and inoculation of all the injuries in one group was made at one time, by sprinkling the roots on the surface of the soil with a suspension of crown-gall bacteria. Inoculated boxes were kept separate from those as yet uninoculated, to avoid the danger of contamination of the injuries with crown-gall bacteria before the desired time interval had elapsed. The results of the experiment are shown in table 5. This experiment was repeated under like conditions the following season, and the time was extended to 50 days. Essentially the same result was obtained as in all earlier experiments (lowest curves, fig. 2).

TABLE 5.—*Length of time during which crown-gall infection was secured through injuries made on roots of red raspberry plants in early autumn,*[a] *1929*

Treatment and date	Period between injury and inoculation	Injuries treated	Injuries infected	
			Oct. 3	Nov. 26
Injuries inoculated[b]	*Days*	*Number*	*Percent*	*Percent*
Sept. 3	1	40	75	85
Sept. 6	4	50	40	64
Sept. 23	21	50	0	42
No inoculum applied		100	0	0
Control injuries made and inoculated				
Sept. 3	0	10	70	90
Sept. 6	0	10	60	90
Sept. 23	0	10	0	70
Oct. 4	0	31	[c] 10	71
Control needle-thrust inoculations				
Aug. 22-Sept. 3	0	90	87	96
Oct. 4	0	31	[c] 7	77

[a] The injuries were made on Sept. 2 on the roots of healthy crown-gall-free plants grown in steamed soil in root-observation boxes.

[b] A suspension of crown-gall bacteria was sprayed over the root and the soil in the root-observation boxes.

[c] Observed on Oct. 24.

The data obtained from each experiment are presented in figure 2. The curves show that the percentage of treated injuries that became infected decreased as the interval between the time of injury and the application of inoculum increased. On vigorous plants this decrease was at first considerable, but after 10 days to 3 weeks the percentage of infected injuries tended to remain constant, indicating probably that a fair proportion of such injuries were rapidly closed to infection but that a small part of the injuries remained subject to infection for 3 to 7 weeks or longer.

A study was made to determine whether injuries on the underground parts of a variety more resistant to crown gall would be subject to invasion by the parasite for a shorter time. Latham and the supposedly more resistant variety Ranere were used. The experiment was carried out on each variety under the same conditions and in the same manner as the experiments described above. The results are recorded in figure 2. The erratic results obtained in the 1930 season may have been due in part to the low vitality of the plants used, a condition that probably resulted from late planting. The results indicate, as did those of all earlier experiments, that injuries to the underground portion of the Latham red raspberry remain subject to crown-gall infection for a considerable period. The comparative record on the more resistant variety, Ranere, suggests that in this respect there is little difference in these red raspberry varieties.

Ranere, often called St. Regis, has been reported resistant and Cuthbert susceptible to crown gall. Comparative studies by the present writer of the development of crown gall in five varieties of raspberry grown during the 1930 season in inoculated sandy-loam soil indicate that Ranere displays some resistance to the disease (table 6). In this experiment the root systems of the Cuthbert plants used were but one third as extensive as those of the Ranere. Had the plants been equal in size the difference between the two varieties in all probability would have been more sharply defined. The Latham variety was not intentionally used in these tests. A few plants developed from roots left in these plots from an earlier planting.

TABLE 6.—*Development of crown gall on 5 varieties of raspberry grown in inoculated soil, 1930*

Variety	Total plants grown	Average galls per plant	Plants infected	Plants visibly injured by root-feeding arthropods
	Number	*Number*	*Percent*	*Percent*
Cuthbert	90	7.0	98	62
Cumberland	52	6.6	94	85
Latham	8	6.6	88	75
Plum Farmer	48	4.9	75	77
Ranere	84	3.2	72	39

Injuries are inevitably caused to the root systems of raspberry plants in storage and in transit. To determine whether injuries to the roots caused prior to planting are subject to infection, 12 plants received from a nursery were injured just before they were planted in the inoculated soil plots described under the heading Seasonal development (p. 779). The injuries were marked by loosely encircling the stem or root at the point of injury with an aluminum wire band. Inspection 14 weeks after planting showed that 19 of the 48 injuries (40 percent) so treated had become infected. Of the 71 injuries made on plants set out in plots of soil that had been steamed none became infected.

INFLUENCE OF MECHANICAL INJURY

Experiments were made to determine whether crown-gall bacteria enter the red raspberry only through injuries. Under controlled conditions in the greenhouse experiments, crown-gall infection did not occur in the absence of either of two factors; namely, (1) injuries to the underground parts of the plant and (2) crown-gall bacteria in the soil in which the plants were grown. Of plants grown in inoculated soil and not guarded against injuries to their underground parts, 80 percent developed the disease. The average number of infections on these diseased plants under controlled conditions was about equal to that found for diseased plants grown in inoculated soil in the field.

These experiments were carried out in the greenhouse on a limited number of plants known to be practically free from crown-gall infections. The plants were disinfected by immersion in silver nitrate solution (1:1,000) for 20 minutes and then planted in 10- or 12-inch pots or in root-observation boxes in soil that had previously been steamed. Prior to the time of the experiment the plants had been grown for 1 or 2 years in the steamed-soil plots previously described. During this period the incipient stages of infection developed that were carried on the plants from the nursery. By discarding the infected plants and selecting only those that had grown at a distance of 3 feet or more from them, plants were obtained having a minimum of incipient macroscopically nonvisible infections. The extent of this minimum quantity of infection was ascertained at the close of each experiment by recording the number of infections found on plants that had not been exposed to crown-gall bacteria during the course of the experiment. Tables 7 and 8 show that no infections were present on the plants selected 1 year after planting in steamed soil, but that a small number of such infections were present on plants removed at the end of 2 years from the steamed-soil beds.

The plants were guarded against the effects of mechanical injury of two types: (1) Injury received when the plants were transplanted at the beginning of the experiments and (2) injury caused by root-feeding arthropods. The injury received in transplanting was practically eliminated as a factor in infection by holding the steam-sterilized soil in which these plants were grown free from crown-gall bacteria for 3 or 4 months. A few injuries received in transplanting became infected when the soil was inoculated 3.5 months after the time of transplanting. Practically, however, this procedure furnished an adequate control against this kind of infection. Injury caused by root-feeding arthropods was guarded against in the 1927-29 experiments by setting the pots or boxes on planks held 3 feet above the greenhouse floor by wooden supports 4 inches square. The boxes or pots were held three fourths of an inch above the surface of the planks by wooden supports 1 by 2 inches. In the experiments conducted in the 1930 season, protection against arthropods was attained by steam sterilization of the soil used, disinfection of plants and containers, and enclosing the plants and their containers within cheese-cloth cages. The plants that were not guarded against root-feeding arthropods were held on the ground (covered by a dense growth of weeds and sod) outside the greenhouse or on the soil floor within the greenhouse.

In the experimental procedure the three main groups of plants were subjected to the variations in conditions shown in tables 7 and 8.

Table 7.—*Influence of (1) injuries caused chiefly by root-feeding arthropods and (2) the presence of crown-gall bacteria in the soil on crown-gall infection of red raspberry, 1927–29*

Total plants	Injuries caused by		Crown-gall bacteria in soil	Infections per plant (average number)
	Transplanting	Root-feeding arthropods		
36	Present	Present	Present	6.1
18	do	do	None	0
25	None	None	Present	2

Table 8.—*Influence of injuries caused chiefly by root-feeding arthropods on development of crown gall on red raspberry plants grown in inoculated soil in 1930*

Plants grown	Conditions of growth		Injuries caused by		Crown-gall bacteria	Galls per plant (average number)
	Place	Time of transplanting	Transplanting	Root-feeding arthropods		
274	Field	Early [a]	Present	Present	Present	4.8
47	do	Late [b]	do	do	do	2.5
27	Greenhouse	do	do	do	do	2.4
39	do	do	do	do	None	.3
91	do	do	None	None	Present	2

[a] For a more detailed record of these plants see table 6

[b] Latham plants were used in all the greenhouse series and in the group of plants transplanted late in the field.

The results of the first series of experiments made in the seasons of 1927 to 1929 are summarized in table 7. Infection was present only on those plants subjected to both injury and the crown-gall bacterium. Four infections occurred on plants subjected to inoculum and guarded against mechanical injury caused by arthropods and against the effect of injury received in transplanting. These galls were 3 mm or less in diameter when the plants were harvested, and occurred at points of contact of root surface and the glass sides of the root-observation boxes used in the experiment. It is probable that these infections took place through minute abrasions of the root surface caused by sand grains. When pressure was applied to the doors covering the glass sides of the boxes, sand grains might have been forced into the roots at these points of contact. Infection occurred in 90 percent of the injuries caused for control purposes on the underground parts of these plants just prior to the inoculation of the soil. Of 201 injuries caused to these plants at various intervals up to 21 days prior to the application of inoculum to the soil, 134 were galled at the close of the growing season. From the ready response of the plants to inoculation and of the injuries caused on these plants to infection (table 5) it is apparent that the lack of infection at other points than those intentionally injured or inoculated could not be attributed to the late application of inoculum to the soil in which the plants were grown. The results of these experiments indicate that there are no natural openings through which infection takes place in early autumn on the underground parts of the red raspberry.

These experiments were repeated on a larger number of plants in 1930. The methods used and the results obtained were essentially the same (table 8). The first set of plants used in this experiment was stunted by lack of adequate drainage in the new type of root-observation box used, and a second series was planted in the same boxes in early June after this defect in construction had been corrected. The smaller amount of infection on the plants in this series subjected to both injury and crown-gall bacteria is believed to have been due to the lower vitality of the plants, which resulted from late transplanting. Under field conditions in inoculated soil the amount of infection that occurred on a number of the same lot of plants was practically equal to that found on the plants exposed to inoculum and arthropods in the controlled series as previously described.

INFLUENCE OF ROOT-FEEDING ARTHROPODS

Many observations suggest that root-feeding arthropods cause most of the injury to the underground parts of the raspberry through which crown-gall infection occurs. Injuries caused by such arthropods have been found in every planting of raspberries studied by the writer at Madison, Wis. In some plantings 85 percent of the plants were visibly injured in this way. Crown-gall infection frequently occurred at such points of injury to the underground parts of plants grown in inoculated soil, but most of the injuries did not become infected. The injury appeared to be caused by a number of root-feeding arthropod forms.

The injury found on the plants was of several types, ranging from very minute abrasions of the surface of delicate rootlets to severe pruning of lateral roots at the crown of the plant and decortication of the underground stem and larger roots. The more insignificant types of injury appeared on tiny roots and could be detected with the naked eye only by the most minute and careful examination (fig. 3, *A*). On the smaller roots (2 mm or less in diameter) cortical incisions were frequently abundant. These occurred singly (fig. 3, *L*) or in series close together and appeared as dark brown parallel lines at right angles to the longitudinal axis of the root (fig. 3, *I*, *J*, *N*). Occasionally the smaller roots were decorticated for short distances (fig. 3, *K*) or had small shallow cavities gouged out of their surfaces (fig. 3, *E*, *F*, *G*, *M*, *O*). These lesser injuries were characteristic of those found on plants grown on the low reclaimed soil of the marsh described in an earlier section of this paper. The most conspicuous type of injury was found on plants grown on the well-drained sandy-loam plots and consisted of large shallow cavities gouged out of the larger roots and the underground portions of the stems (fig. 4, *A* to *E*). Besides such injury, many branch roots, often 50 percent or more, were completely severed from the plant (fig. 4, *F*). This type of injury was not observed during the seasons of 1928 and 1929. The majority of the plants grown on the sandy-loam soil in 1930 were injured in this manner (table 6).

Crown-gall infection developed on a large percentage of injuries to the underground parts of red raspberrry plants grown in inoculated soil (figs. 3 and 4). Usually after several months of development the galls had completely obscured the injury through which the infection took place. Gall development obscured within a few weeks the smaller injuries through which infection occurred (fig. 3, *A* to *H*, *N* to *S*). In the seasonal-development study discussed later there was no indication of the manner in which infection occurred in over 95 percent of the galls. The injuries through which infection entered the plants grown in the reclaimed marsh soil were of an inconspicuous type (fig. 3).

In two types of experiment, involving the exclusion and inclusion of arthropods, respectively, it has been shown that root-feeding arthropods cause the injuries through which crown-gall infection occurs on the raspberry. In the exclusion experiments, groups of Latham red raspberry plants were grown in inoculated soil from some of which arthropods were excluded and from some of which they were not excluded. The methods have been already described. When no precautions were taken to exclude root-feeding arthropods from these plants, infection occurred as abundantly as under the usual field conditions. When the plants were protected from such arthropods in the soil in which they were grown no infection was observed (tables 7 and 8). The field observations and experimental results indicate that under ordinary conditions root-feeding arthropods are the chief factor in creating the injuries through which crown-gall infections occur. In these experiments no attempt was made to ascertain what arthropod species caused the injuries in question.

The arthropod-inclusion experiments consisted in confining root-feeding species (1) to limited areas of root surface and (2) to the soil in the container in which the plants were grown. The arthropod forms used were click-beetle larvae (Elateridae), millepedes (Diplopoda), and white grubs (*Phyllophaga* sp.). In the first type of experiment the larvae were confined to short uninjured lengths of lateral roots from 1 to 3 mm in diameter, by enclosing a length of 2 to

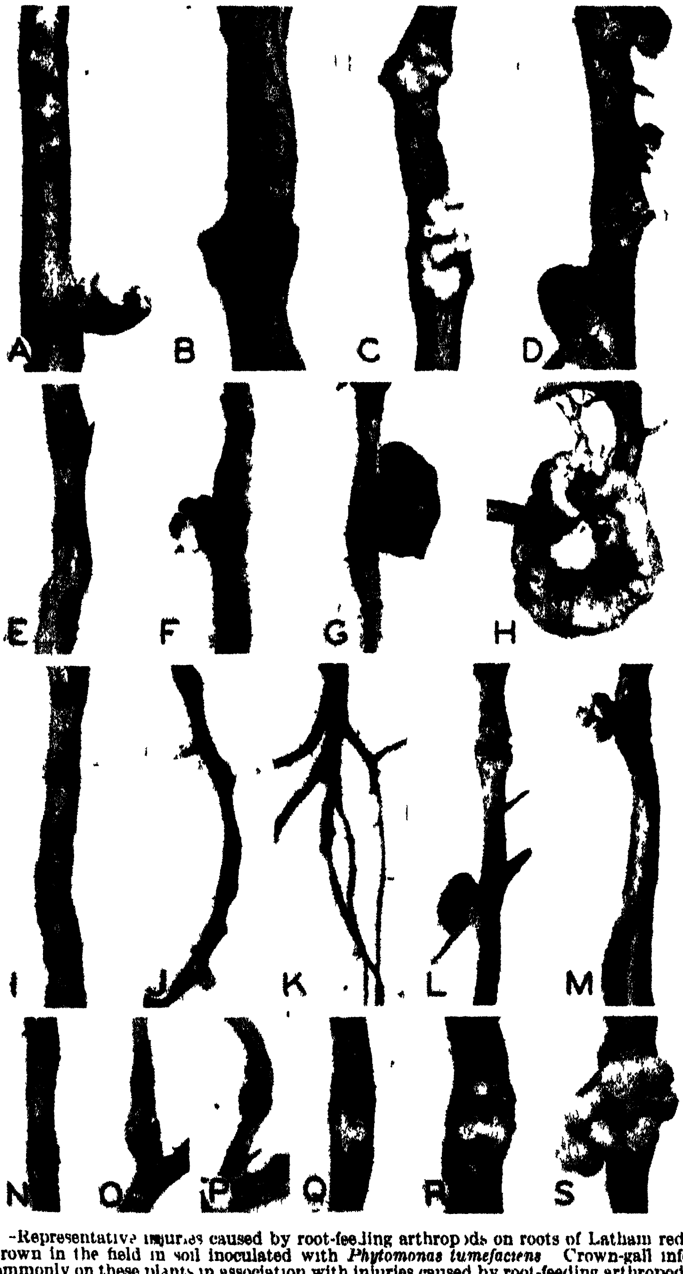

FIGURE 3.—Representative injuries caused by root-feeding arthropods on roots of Latham red raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens*. Crown-gall infection was found commonly on these plants in association with injuries caused by root-feeding arthropods. Crown gall has developed from infection through a number of injuries shown. Injuries: *A*, Minute abrasions of the root cortex (upper half of figure) at top of stem, barely visible to the naked eye, *B*, *C*, *E*, *F*, *G*, *M*, *O*, *P*, small shallow cavities chewed from the roots, *I*, *J*, *N*, multiple incisions in the roots, *K*, decorticated small lateral roots. Infections: *B*, *K*, *Q*, Crown-gall overgrowth just appearing from infection through injuries, *C*, *G*, *O*, *R*, crown-gall overgrowth is rapidly obscuring the injuries through which infection occurred, *D*, *H*, *L*, *S*, lower *A*, *F*, *J*, upper *M*, crown-gall overgrowth has obscured the injury through which infection occurred, *Q*, *R*, *S*, stages of crown-gall formation after infection through injuries caused by a scalpel, *H*, circular depressions have resulted from the feeding of grubs on a crown gall. Scale of enlargement *K*, × 1, *H*, *Q*, *R*, *S*, × 2; *C*, *D*, × 3, *G*, *I*, *J*, *O*, *P*, × 4, *B*, *E*, *F*, *M*, *N*, × 5, *A*, *L*, × 7.

4 inches of one or several such roots in a wire screen. The wire screen used was of brass (100 meshes per square inch), and was sewed with brass wire in the form of a tight sack, 4 by 3 by 1.5 inches, about the roots. The smaller spaces around the points where the roots protruded from the sack were stuffed with glass wool. Two wireworms were put into each of two such wire-screen sacks, which were then

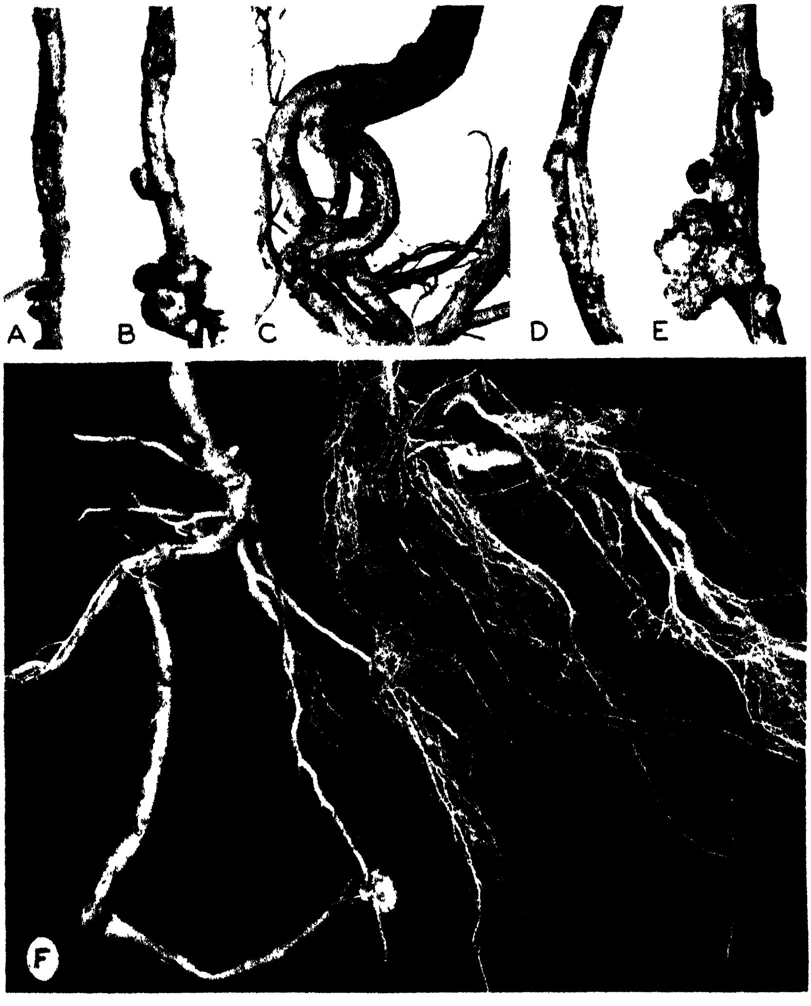

FIGURE 4.—Typical white-grub injury on the roots of raspberry plants. Crown-gall infections were usually associated with this type of injury in 1930 at Madison, Wis., on all raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens.* Injuries: Shallow to deep cavities were gouged by white-grubs, *Phyllophaga* sp., from the larger roots, and the underground portions of the stem of both red (*A*, *B*, *F*) and black (*C*, *D*, *E*) raspberry varieties. Frequently 50 percent or more (*F*, left) of the lateral roots were eaten from plants in field plots. *A*, *B*, Injuries caused by grubs on the roots of Latham red raspberry plants grown in a container of inoculated soil in which white grubs had been placed. Infections, *B*, Crown-gall development at top not perceptible from infections through injury, *A*, *C*, Barely perceptible at the injury; *D*, rapidly covering the injury; and *B*, *E*, obscuring the injury. *F*, Representative root system of a series of plants grown in the greenhouse in pots of inoculated soil and subjected to the following conditions. White grubs were placed in the soil (left) and root-feeding arthropods were excluded from the soil (right). Crown-gall infection has occurred through several of the injuries caused by the grubs. No injuries and no infection occurred on plants from which arthropods were excluded. Scale of enlargement: *A*, *B*, *D*, and *E*, × 2; *C*, natural size; *F*, ½ natural size.

filled with soil and sewed shut. One millepede was put into each of two other screen cages. One white grub was put into each of four similar root enclosures. The soil in the sacks was then inoculated by applying to the soil of the root-observation boxes used a suspension of crown-gall bacteria. Six weeks later the screen cages were removed and observations were made. The roots in one of the wireworm cages were not noticeably injured. In the other, one side of the root enclosed was decorticated for a length of 2 inches and crown-gall infection had developed at two points along this area of injury. Since the injury extended past the end of the screen cage, it is possible that it may have been made in fastening the cage about the root. The millepede in one cage had decorticated the root in two places, no infection had occurred, and the root had died. The white grubs had eaten all the roots enclosed within each of the four screen cages in which they had been confined, leaving not a trace. Of the four, two escaped into the soil in the free space of the root-observation box. Examination of the roots in the box revealed injuries identical in type with those found on the majority of plants grown in the field in 1930 (fig. 4, *A*, *B*). Crown-gall infection had occurred through two of seven such injuries. All the arthropods employed in the foregoing studies, except one millepede, were recovered alive at the close of the study.

A further experiment was made in which white grubs were placed at large in pots in which disease-free raspberry plants were growing. The soil was then inoculated, as was the soil in which a control group of plants were growing. The control group was protected from all arthropod forms. In each group there were ten 6-inch pots, each containing two plants. Six weeks after two large white grubs had been placed in each of the pots of the first group and the soil of both groups had been inoculated with a suspension of crown-gall bacteria, all plants were washed free of soil and examined. Of the 20 plants grown in pots in which grubs had been placed, all were conspicuously injured. Large shallow cavities had been gouged from the underground part of the stem and the larger roots. Frequently the roots had been either chewed completely off or about half eaten away for a length of 1 to several inches. The lateral roots of smaller diameter had been largely eaten away (fig. 4, *F*). Of these 20 plants 7 had developed crown-gall infection at the points of white-grub injury, with an average of 3.6 galls per plant; 2 of the remaining 13 had died. No injuries were found on the underground parts of the plants in the parallel group that had been protected from all arthropod forms, and no crown gall developed on these, though both groups had been exposed alike to inoculum. The injury caused by these grubs was identical with that found on plants grown in the inoculated field plots in which white grubs were found in abundance (fig. 4, *B* to *E*; table 6). From these experiments and observations it is concluded that in 1930 white grubs caused most of the injury through which crown-gall infection developed on raspberry plants grown in inoculated soil at Madison, Wis.

INCUBATION PERIODS

During the studies, already described, on the length of time wounds remain open to infection, variation in the incubation period of crown-gall infections on the underground parts of red-raspberry plants was observed to occur. This variation appeared to be correlated with (1) the general condition of the host plant in response to environmental factors, particularly those relating to the season, and (2) local variation within individual plants due to differences in age and vigor of roots. It was observed that overgrowths of appreciable size appeared within 11 days after inoculations made on August 23 (table 5), whereas inoculations made on October 4 did not induce appreciable overgrowths until 3 weeks later. From the data recorded in table 5 it is clear that many inoculations which had not induced macroscopically visible overgrowths 3 to 4 weeks after the application of inoculum developed such overgrowths later. In these studies the incubation period of crown-gall infections on the roots of the red raspberry was observed to range from 11 days to more than 4 weeks.

A knowledge of the prolonged incubation period, which appears to lengthen as the plants approach the dormant condition, and of the relatively long period in which injuries to the underground parts of the red raspberry may remain subject to infection by crown-gall bacteria, is important in understanding the incipient stages of infection by which the disease is disseminated to new areas.

SEASONAL DEVELOPMENT

Little has been reported heretofore concerning the time at which crown-gall infection occurs, and the course of the development of this disease on the red raspberry has remained obscure. From seasonal development studies reported herein it was found that crown-gall infection occurred (1) at random on all underground parts of the plants and (2) at a practically uniform rate throughout the course of the two seasons studied.

For a period of 2 years a study was made of the seasonal development of crown gall in a planting of Latham red raspberries. All the plants studied were without macroscopically visible infections when they were planted in a field plot of inoculated Clyde silt-loam soil. The plot was inoculated 1 day before planting by mixing into the upper 9 inches of soil a suspension of crown-gall bacteria applied at the rate of 1 pint per square foot of soil. The suspension used was made and applied as described earlier for the overwintering studies. From time to time during the season in which these plants were set out and during the following season a number of plants were dug at random from the plot and their condition with regard to the disease was recorded. Each plant was washed free of soil particles in a stream of water, and all its parts were then examined minutely for macroscopically visible infections. In this way all visible galls on the plant were recorded with notations as to their size and position. From 40 to 75 plants were examined and discarded at each observation. The data obtained from this study are presented in condensed form in tables 9, 10, and 11, and in figure 5.

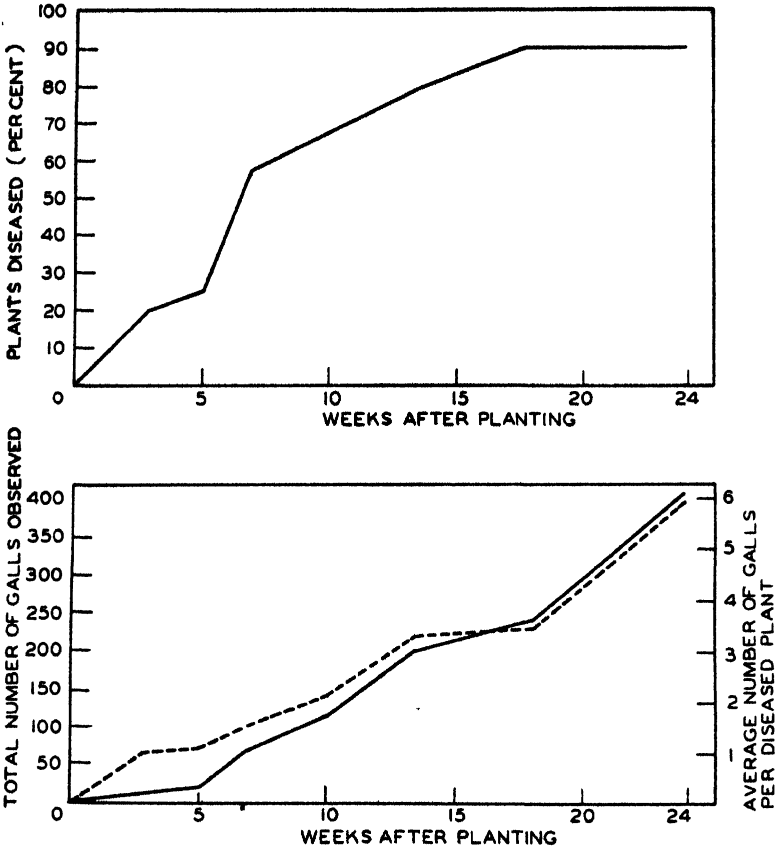

FIGURE 5.—Seasonal development of crown gall on underground parts of red raspberry plants grown in the field in soil inoculated with the crown-gall organism. Broken line in lower graph indicates average number of galls per diseased plant.

TABLE 9.—*Seasonal development of crown gall on underground parts of red raspberry plants grown in inoculated soil, 1928–29*

Date	Length of time after planting	Plants examined		Galls present					
						Distribution according to indicated diameter			
		Total	Infected	Total	Average per infected plant	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm
1928	*Weeks*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
June 6	3	40	20	8	1.0	3	2	3	0
June 23	5	40	25	11	1.1	8	1	2	0
July 6	7	75	56	68	1.6	49	8	7	6
July 27	10	40	68	61	2.2	28	15	11	7
Aug. 17	13	75	80	218	3.6	54	67	68	29
Sept. 17	18	75	91	247	3.6	36	83	61	67
Oct 28	24	75	92	402	5.8	66	99	105	132
1929									
June 1	55	50	90	285	6.3	34	59	133	43
Aug. 1	64	50	94	[a] 271	5.7	3	54	110	34
Nov. 1	78	50	96	302	6.3	12	24	180	86

[a] The total number of galls here includes 70 which had disintegrated to the stage where they could be detected only by scars left on the roots.

TABLE 10.—*Size and location of crown galls observed during the first season on underground parts of red raspberry plants grown in inoculated soil, 1928* [a]

Length of time after planting (weeks)	Location and size of crown galls											
	Galls of indicated diameter on stem				Galls of indicated diameter on branch roots larger than 2 mm				Galls of indicated diameter on branch roots less than 2 mm			
	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm
	No.	*No.*	*No.*	*No*	*No.*	*No.*	*No*	*No*	*No*	*No*	*No.*	*No*
3	2	2	2	0	1	0	1	0	0	0	0	0
5	2	1	2	0	2	0	0	0	4	0	0	0
7	6	2	3	3	2	4	3	2	41	2	1	0
10	0	1	5	4	7	11	4	3	21	3	2	0
13	2	10	17	10	21	20	25	15	31	37	26	4
18	0	11	14	19	6	24	25	43	30	48	22	5
24	4	12	10	39	16	37	64	74	46	50	31	19

[a] For dates of observation, number of plants examined, number of infected plants, and total number of galls observed, see table 9.

TABLE 11.—*Size and condition of crown galls observed during the second season on underground parts of red raspberry plants grown in inoculated soil, 1929* [a]

Date of observation	Condition of galls observed	Total galls observed	Average galls per plant	Distribution of galls according to diameter			
				0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
June 1	Living	132	2.9	28	34	53	17
	Dead	153	3.4	6	25	78	44
Aug. 1	Living	123	2.6	2	41	57	23
	Dead	[b] 148	3.1	1	13	53	11
Nov. 1	Living	170	3.5	12	19	107	32
	Dead	132	2.8	0	5	73	54

[a] The number of plants examined, number of diseased plants, and the average number of infections per plant are given in table 9.

[b] 70 galls which had disintegrated to the stage where their presence could be distinguished only by the scars that their decay had left on the roots could not be recorded as to size.

As an ecological check, two smaller plots of Miami sandy loam were inoculated and planted at the same time as the silt-loam plot described above. These smaller plots were situated on a gentle slope about 1 mile distant from the drained low marshland on which the silt-loam plot was located. The same soil treatments and the same plant materials were used in both experiments. The plants in the smaller plots were not examined until the close of the second season, however, at which time all were harvested and a detailed record was made of their condition with reference to the disease. The comparative record of the development of crown gall on plants grown in these inoculated sandy-loam and silt-loam soils is shown in table 12.

TABLE 12.—*Comparison of crown galls found on red raspberry plants grown in sandy-loam and in clay-loam inoculated soils* [a]

Age of plants and soil type	Plot no.	Total plants	Infected plants	Total galls	Average galls per infected plant	Distribution according to diameter of crown galls observed							
						Living				Dead			
						0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm
2-year plants:		*No.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No*	*No.*	*No.*	*No*	*No.*
Sandy loam	22	37	95	285	8.1	32	28	94	31	0	2	49	49
Do	21	34	94	[b] 283	8.8	51	53	118	61				
Clay loam	M	50	96	302	6.3	12	19	107	32	0	5	73	54
1 year plants.													
Sandy loam	22	28	57	35	2.2	14	6	9	6	0	0	0	0
Do	21	36	67	[c] 131	5.4								
Clay loam	M	94	17	22	1.4	3	2	13	4	0	0	0	0

[a] Data were recorded at time of harvesting the plants at close of second growing season subsequent to planting in inoculated soil

[b] No record was made of the condition of these galls

[c] No record was made of the condition or size of these galls.

A further check to ascertain the number of undeveloped infections that these plants carried when shipped from commercial nursery storage was made in parallel plantings on sandy-loam plots that had been steamed. These plots were at a slightly higher level and were adjacent to the inoculated sandy-loam plots described above. The development of the disease on the plants in these steamed plots has been discussed already. All plots were planted from a shipment of Latham red raspberry plants received from a nursery in May 1928.

The following is a summary and brief discussion of the results of the study of the seasonal development of crown gall on red raspberry plants grown in inoculated soils.

Infection occurred at all times and at a practically uniform rate throughout the entire growing season.

The number of plants showing crown-gall infection increased rapidly from 20 percent in early June of the first season (an increase at that time of 5 percent over the controls planted in the steamed beds) to about 90 percent by the middle of September. The number of diseased plants then gradually increased to 96 percent by the close of the second season (table 9, fig. 5).

The number of infections per plant increased steadily and at a practically uniform rate throughout the growing season. This increase appears as a relatively uniform progression when the number of infections is plotted against time (fig. 5). The average number of infections found per diseased plant in June was 1.0 as contrasted with 5.8 at the close of the season. In June of the second season a maximum of 6.3 infections per diseased plant was reached. Although new infections continually appeared during the second season (table 11) the number of galls found per diseased plant remained about constant. This may have been due in part to decomposition of galls formed as a result of infections that occurred during the first season.

Infection appeared to occur at random on all underground parts (fig. 6). It did not seem to be confined to any particular developmental phase or portion of the plant. On the underground section of the stem, on the branch roots of large or small diameter, infection

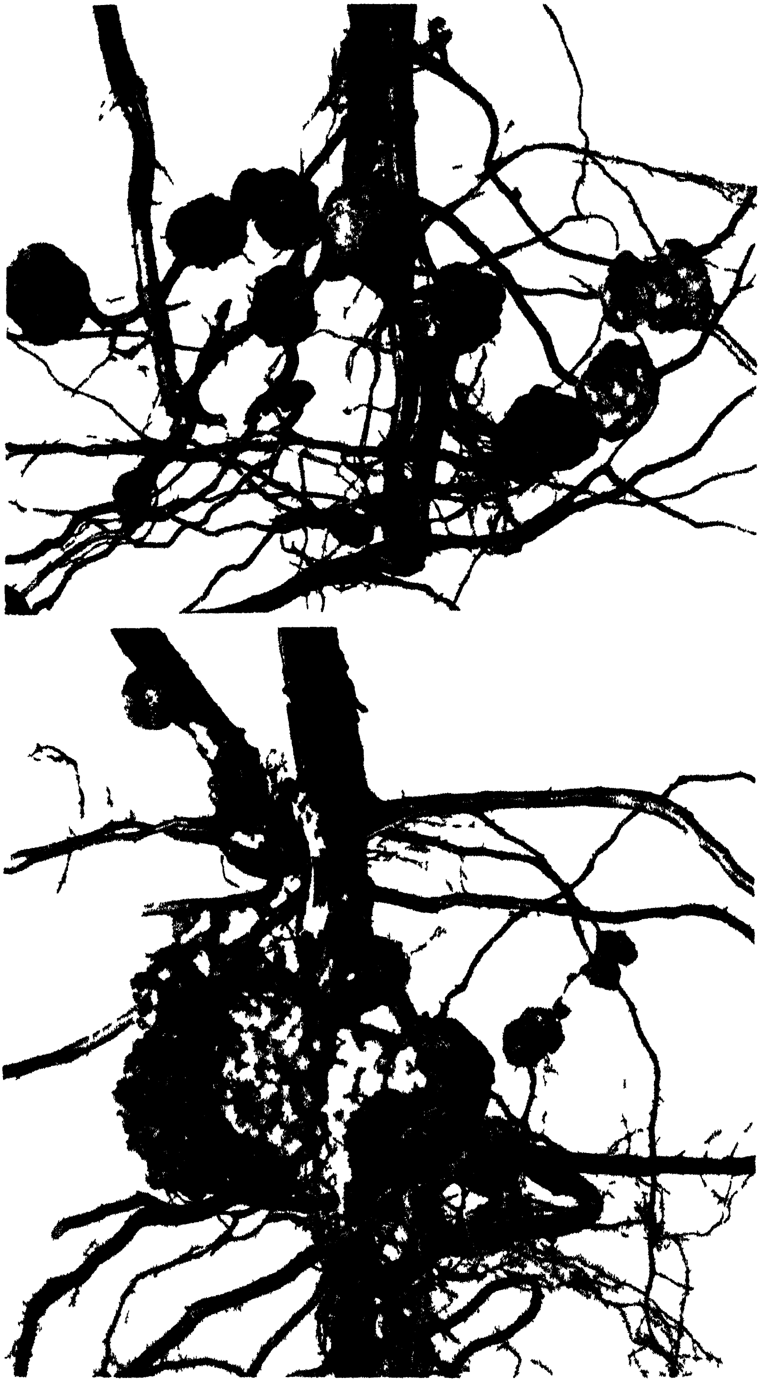

FIGURE 6.—Typical crown gall on the roots of Latham red raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens*. This type of crown gall occurred on the roots of over 95 percent of the plants grown under these conditions. Aerial portions of the plants were affected very rarely; less than 0.2 percent of all plants grown.

occurred alike and continuously throughout the season (table 10) As discussed in an earlier section, infection appeared with greatest frequency on those underground portions of the plant within 4 inches of the surface of the soil.

Infection was repeatedly observed to have taken place through injuries caused by root-feeding arthropods.

The disease developed more extensively on plants grown on the higher well-drained sandy loam (table 12) than on plants grown on the reclaimed silt loam of the marsh. At the time of harvesting, the disease seemed from general observation to be more severe on the plants grown in the sandy loam. That the disease did develop more extensively on the plants in these plots is apparent from the comparative record (table 12), from which it is shown that:

The number of first-year plants diseased was 57 to 67 percent, respectively, for the sandy-loam plots and 17 for the silt-loam plot; the average number of infections per diseased first-year plant was 2.2 and 5.4, respectively, for the sandy-loam plots, and 1.4 for the silt-loam plot; the average number of infections per diseased 2-year plant was 8.1 and 8.8, respectively, for the sandy-loam plots and 6 3 for the silt-loam plot; toward the end of the second season the rate at which infection was occurring in the sandy-loam plots was about three times that in the silt-loam plot. This rate is a function of the number of 0.5 to 2.0 mm galls found on the plants at any observation.

SUMMARY

Crown-gall bacteria are given off continuously from the surface of living crown galls under suitable moisture conditions

Crown-gall bacteria were found to overwinter in the soil of fallow fields at Madison, Wis., and to exist in a pathogenic state for 14 months in unsterilized soil held free from the growth of seed plants under nursery storage conditions

Crown gall is carried from the nursery to new areas as incipient undeveloped infections on the raspberry that cannot be detected by inspection.

Crown-gall infection was found to occur at a practically uniform rate throughout the course of the growing season on red raspberry plants grown in inoculated soil.

Crown-gall infection occurs on the raspberry only through injuries No infection was found on plants which were exposed to inoculum but the underground parts of which were protected from injury.

Injuries on the underground parts of the red raspberry remain subject to crown-gall infection for a relatively long period A large percentage of the injuries studied became infected when inoculum was applied to their surfaces 3 weeks after the injury was made. A small percentage of those studied became infected when inoculum was applied to them 7 weeks after the injury was made.

The incubation period of crown-gall infection on the underground parts of the red raspberry was found to vary from 11 to more than 28 days as a result of environmental conditions, chiefly seasonal.

In these investigations root-feeding arthropods caused most of the injuries through which crown-gall bacteria entered. The injuries found on the underground parts of the plants probably were caused by a number of species of arthropods. White grubs caused most of the injuries through which infection occurred in 1930.

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CHROMOSOMES IN HYBRIDS BETWEEN EUCHLAENA PERENNIS AND ZEA MAYS[1]

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INTRODUCTION

Hybrids between maize (*Zea mays* L.) and perennial teosinte (*Euchlaena perennis* Hitchc.) offer a favorable opportunity to follow the behavior of triplicate sets of chromosomes in a triploid hybrid. Maize, the diploid parent, has been carefully analyzed both genetically and cytologically. Perennial teosinte seems to be unquestionably a true tetraploid. Its tetraploid character is evident from (1) the prevalence of tetravalent chromosomes at diakinesis observed by Randolph[3] and by the writer; (2) the X-ray experiments of Randolph (*17*),[4] who obtained from the 20-chromosome annual *Euchlaena* a 40-chromosome perennial plant very like *E. perennis*; and (3) genetic studies which show that F_1 teosinte-maize hybrids contain three sets of homologous chromosomes. Although extensive genetic studies were carried on in conjunction with the cytological studies, the present paper treats only of the chromosomes as found in F_1 and later hybrid generations.

The first plants of perennial teosinte were found in Mexico by Hitchcock in 1910 and were introduced into this country by Collins (*4*) in 1921. The first hybrid with maize was made early in 1922 by C. G. Marshall. The breeding has been done largely at the United States San Diego Acclimatization Garden at Torrey Pines, Calif., where the more promising perennial hybrid forms are maintained in permanent plantings. The methods of crossing have been similar to those used in corn-hybridizing studies, but to bring plants with very different flowering dates into flower at the same time, plantings were made at different seasons and late-flowering plants were frequently given an artificial short day.

The cytological material, which had been collected just before the tassel emerged from the leaves, was put in Carnoy's killing fluid for half an hour; this fluid was then poured off and the material was covered with absolute alcohol. Material preserved in this way is generally satisfactory for the determination of chromosome numbers by the use of the acetocarmine smear method.

CHROMOSOME BEHAVIOR IN POLLEN MOTHER CELLS

The hybrids between maize and teosinte that were used in this study were F_1, F_2, back crosses, and more complicated crosses. The determination of the chromosome numbers of individual plants was

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[3] Unpublished manuscript.

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most readily made at the first-division anaphase. Figure 1, *A–D*, shows this phase in an F_1, a back cross, a back cross again backcrossed to maize, and a self of a back cross, respectively.

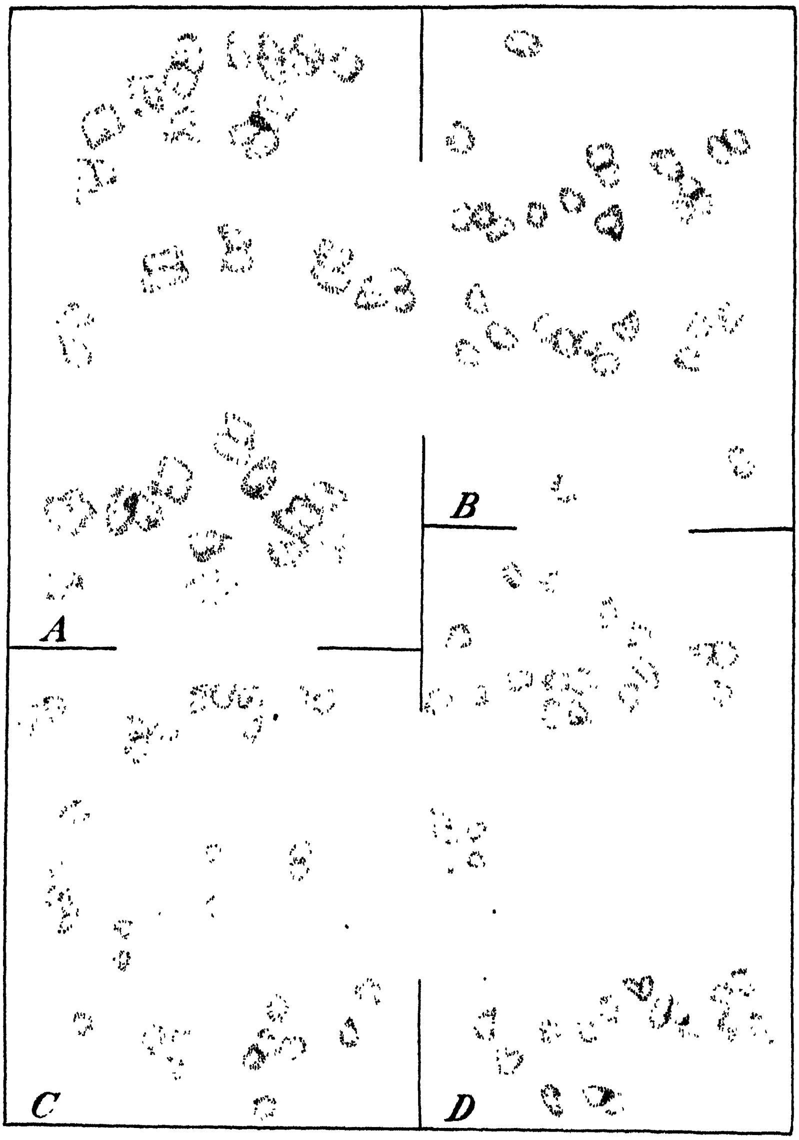

FIGURE 1.—First-division anaphases in teosinte-maize hybrids: *A*, 30 chromosomes of an F_1 plant; *B*, 29 chromosomes of a back cross; *C*, 28 chromosomes of a back cross crossed with maize, *D*, 35 chromosomes of a selfed back cross *A* × 1,900, *B–D* × 1,200.

At this phase all bivalent chromosomes have divided and the univalents appear almost identical with the halves of the bivalents, so that a count of all chromosomes gives at once the $2n$ number of the plant under inspection. If the figure happens to be in late anaphase, a few of the univalents may be in the region of the plate in the process of division. Even at this stage the character of the dividing univalents is such that they are not mistaken for bivalent chromosomes.

In 1924 the writer (*13*) reported the number and some of the characteristic features of the chromosomes in the developing pollen mother cells of F_1 plants of crosses between *Euchlaena perennis* and *Zea mays.* Since that time additional material has been available, and it has been found that with one exception, referred to later, all plants have 30 chromosomes, the sum of the haploid chromosome numbers of the two parents.

The behavior of the chromosomes in these hybrids during meioses of the pollen mother cells has been found to be that already described (*13*), except in a few minor details. A closer examination of the disposition of the univalent chromosomes, which are usually scattered on the spindle at the time the bivalent and trivalent chromosomes form the first-division metaphase plate, shows that many of them move undivided to the poles with the halves of the divided chromosomes. However, one or more of the univalents frequently will be found at the plate region when the divided chromosomes are in the anaphase, and at late anaphase these univalent chromosomes will divide. Except for the occasional undivided univalents or halves of univalents that are too far afield, the two daughter nuclei include the divided halves of the bivalent chromosomes and varying numbers of divided and undivided univalent chromosomes. Those chromosomes that fail to be included in the daughter nuclei are left in the cytoplasm to form micronuclei or to degenerate.

The second division is much more regular than the first. Only the halves of univalents present cause any appreciable abnormalities. Their position on the metaphase spindle is indefinite and their distribution is at random to the two poles.

The chromosome behavior during meiosis of the pollen mother cells of later hybrid generations is in most respects similar to that observed in F_1 hydrids. The chromosome numbers in F_2, in back crosses, and in others of the more complicated hybrids are likely to vary for each individual. The chromosome complement of such plants will be made up of chromosomes derived from the two parents. The number from either parent is unpredictable, and unfortunately cytological study has not yet developed any method of differentiating the chromosomes of maize from those of teosinte.

Camera-lucida drawings were made as a record of the chromosomes of each plant, since the temporary slides could not be preserved for any appreciable time. The drawings of first-division anaphases gave an opportunity to study the distribution of chromosomes to the two daughter nuclei resulting from the first meiotic division.

The distribution of the chromosomes in an F_1 hybrid seems generally to show that of the 3 sets of 10 chromosomes present 2 sets pair regularly and 10 chromosomes go to each pole, whereas the chromosomes of the third set are distributed at random to the two daughter nuclei. Figure 2, *A*, shows 10 bivalents, 1 pair of loosely associated chromosomes, and 8 univalents. In plants of later hybrid generations, however, the number of regularly pairing chromosomes may be more than 10, since a plant may have 4 or more homologous chromosomes instead of just 3 as in F_1 hybrids. Figure 2, *B* and *C*, shows cells having 22 chromosomes. In *B* all chromosomes are paired, and in *C* there are 10 pairs and 2 univalents, suggesting that in the former there are 4 homologous chromosomes present, whereas in the latter the 2 unpaired chromosomes are not homologous.

The genetic data have shown that in F_1 plants and in back crosses on maize autosyndesis is the rule and allosyndesis the exception, so that in F_1 hybrids the unpaired chromosomes are from maize, whereas in plants from back crosses on maize the unpaired chromosomes are from teosinte.

Table 1 gives a comparison between the numbers of chromosomes that go to make up the daughter nuclei resulting from the first meiotic division of 30-chromosome hybrids and the numbers calculated when 10 paired chromosomes go at random to join the 10 divided bivalents at the two poles, i.e., the binomial expansion of $(a+b)^{10}$. Although the data of this table are made up from drawings of plants with different percentages of maize and teosinte chromosomes, it indicates that the number of chromosomes between 10 and 20 that enter a gamete depends upon chance. The binomial expansion does not represent the chance distribution of extra chromosomes in any generation following F_1, for then the number of pairs is not limited to 10. For example, in a plant with 30 chromosomes it is possible to have 15 paired and no unpaired chromosomes, and in such a plant the distribution of the chromosomes at the first meiotic division invariably will be 15 chromosomes to each daughter cell. To calculate an expected chance distribution of chromosomes in 30-chromosome plants of perjugate generations it is necessary first to calculate the expected chance distribution of additional pairs and then apply a binomial distribution to the remaining unpaired chromosomes. Distributions have been calculated for plants with 22, 23, and 24 to 40 chromosomes, based on random distribution of the extra chromosomes from an unlimited number of sets of 10 chromosomes. The observed distributions of chromosomes in many of the plants with a few extra chromosomes are sufficiently close to the calculated distributions to verify the assumption that in later generations the distribution approaches this modified distribution more nearly than it does the simple binomial expansion of the number of chromosomes above 20 present in an individual. However, agreement with such a modified distribution of extra chromosomes does not conflict with

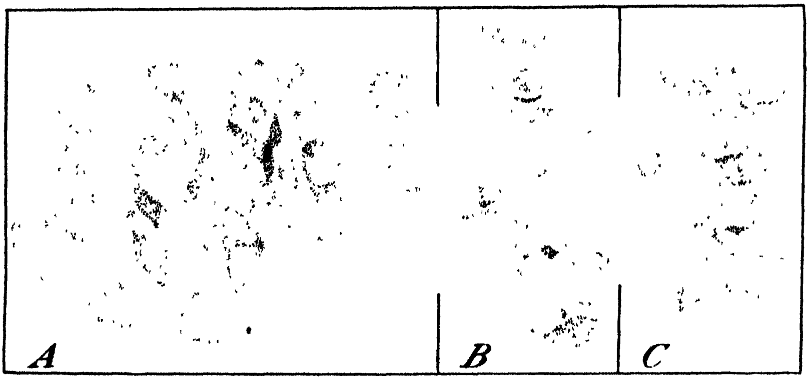

FIGURE 2.—First-division metaphase of teosinte-maize hybrids *A*, 11 bivalent and 8 univalent chromosomes of an F_1 plant, *B*, 11 bivalent chromosomes of a later hybrid generation, *C*, 10 bivalent and 2 univalent chromosomes from a sister plant of *B* *A* × 1,800; *B* and *C* × 1,200

the statement that univalent chromosomes are distributed at random, but is simply a more exact method of estimating the number of unpaired chromosomes.

TABLE 1.—*Distribution of chromosomes to the 2 daughter nuclei resulting from the first meiotic division of 30-chromosome hybrids*

Chromosomes	Number of cells with indicated chromosome distribution					
	15–15	16–14	17–13	18–12	19–11	20–10
Observed	17	30	14	9	5	0
Calculated $(a+b)^{10}$	18.4	30.8	17.6	6.6	1.5	00+

The data presented in table 1, supported by similar data from drawings of chromosomes of hybrids having chromosome numbers

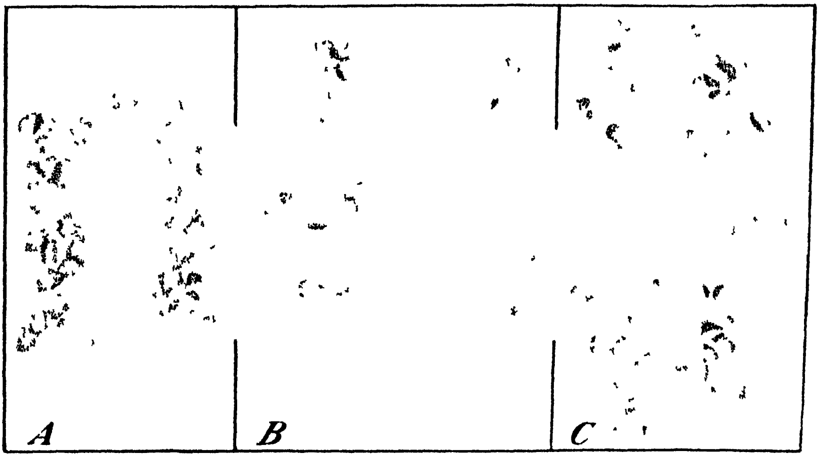

FIGURE 3.—First division anaphases of teosinte-maize hybrids with aberrant chromosome numbers *A*, 40 chromosomes from an F_1 plant, *B*, 42 chromosomes from a plant of a progeny twice back-crossed on maize, *C*, 56 chromosomes from an F_2 plant × 1200

other than 30, indicate that there is a random distribution of all unpaired chromosomes and that gametes are produced with all chromosome numbers in approximately the predicted numbers in each group, the number of chromosome groups depending upon the number of univalent chromosomes present to be distributed at random.

ABERRANT HYBRID PLANTS

In a progeny of 7 F_1 hybrids grown in 1932, it was noticed that 1 plant was markedly different from the 6 sister plants or from any of the previously grown F_1 hybrids. The ears were generally 4-rowed, the pollen showed very little sterility, and abundant seeds were produced after either cross-pollination or selfing. This plant was found to have 40 chromosomes (fig. 3, *A*). The fact that it seemed more cornlike than other F_1 plants suggested that this increase of 10 chromosomes above that of normal F_1 plants was due to the presence of two sets of corn chromosomes. Perhaps the most logical assumption to account for the double number of chromosomes is that doubling took place soon after fertilization; but other possibilities, such as double fertilization, make it unwise to speculate.

Emerson and Beadle (*6*) have described a similar aberrant F_1 plant, and the present plant agrees very closely with their description. Such plants, as they suggest, may offer possibilities for genetic and cytological studies.

A small F_2 population from the aberrant plant was grown. The plants were very uniform in appearance, and chromosome counts showed that all of them had 40 or approximately 40 chromosomes.

A second aberrant plant appeared in a progeny twice back-crossed with maize. Sister plants of this progeny were found to have chromosome numbers ranging from 20 to 28, whereas this plant had 42 chromosomes (fig. 3, *B*). Such a high number suggests a doubling of all chromosomes after fertilization. Although all the experimental plants resulted from guarded pollination, outcrossing might explain the high number of chromosomes; but such an explanation is unlikely, since the plant had 14 more chromosomes than were found in the sister plant having the next highest number. To produce a plant with 42 chromosomes would require a functioning pollen grain with 24 or more chromosomes, a number exceptionally high for the pollen of any hybrid that has yet been grown. The presence of the *wx* gene further reduced the probability that the plant resulted from outcrossing, since no plants were being grown that could produce pollen grains having high numbers of chromosomes and the *wx* gene.

Two plants, one with 56 chromosomes (fig. 3, *C*), from the F_2 progenies of teosinte and maize, have had high chromosome numbers that suggested a doubling of the chromosomes contributed by either parent or by both. The occurrence of these plants with high chromosome numbers is in keeping with the finding of similar abnormal hybrid plants by investigators of other plant groups.

CHROMOSOME MORPHOLOGY

The investigations of McClintock (*16*) and Beadle (*1*) have stimulated an attempt to search the midprophase of some of the hybrid plants, since in many forms there are three allelomorphic chromosomes and often it would be very useful if such chromosomes could be identified. A few plants having only 21 chromosomes are known to be trisomic for chromosome no. 9, the chromosome that carries the *wx* gene, and it seemed that such plants would be useful for a preliminary study. This study, however, has proved disappointing, partly because of the lack of any definite morphological knowledge of the chromosome complement of *Euchlaena perennis*. It has not been difficult to identify chromosome no. 5 (of *Zea*) by its characteristic attachment to the nucleolus. A short chromosome with a terminal knob on the short arm was quite frequently lying where it could be traced and drawn. This chromosome resembles McClintock's drawings of chromosome no. 9, but Beadle (*1*) has shown that terminal knobs are prevalent on the chromosomes of Florida teosinte. It seems unsafe, therefore, to ascribe a definite identification to such a chromosome in these hybrid plants.

TABLE 2.—*Chromosomes in back-crossed and F_2 Euchlaena perennis–Zea mays hybrids*

Female parent		Male parent		Number of plants with indicated chromosome number																								Mean chromosome number		
Species or cross	Chromosomes	Species or cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total plants	Observed	Expected	Of functioning gametes of hybrid plant
	No.		*No.*																								*No.*			
E. perennis × *Z. mays*	30	*Z. mays*	20	1	1	1	1	4	5	5	5	2		1													26	25.3	25	15.3
Z. mays	20	*E. perennis* × *Z. mays*	30									4	24	66	8												102	29.8	25	19.8
E. perennis × *Z. mays*	30	do	30						1	1	1			1	1	3	5	14	23	29	31	19	5	5		1	140	35.94	30	

A disconcerting observation of the threads of midprophases was the absence of univalent threads in plants known to have one or often several univalent chromosomes. Beadle (*1*) has referred to a similar absence of univalent threads in hybrids of Florida teosinte and maize. A further study of what seems to be very suitable material is being made in an attempt to definitely trace the behavior of the univalent chromosomes in early prophase stages.

CHROMOSOME SELECTION IN GAMETES

The study of the chromosome numbers prevalent in the functioning pollen and ovules of any particular teosinte-maize hybrid is most readily determined by making reciprocal crosses on maize in which the gametes are known to have 10 chromosomes. By subtracting 10 chromosomes from the chromosome numbers of the plants of the back-crossed progeny, the chromosome number of the gametes of the hybrid parent is obtained. The data of tables 2, 3, and 4 summarize the observed chromosome numbers of the plants of these and other more complicated progenies.

The data of table 2 give the chromosome counts of 26 back-crossed plants in which the F_1 was the female parent, of 102 in which the F_1 was the male parent, and of 140 F_2 plants.

The mean chromosome number of the 26 plants used to determine the chromosome number of the functioning ovules of F_1 plants is 25.3. If 10 is subtracted, the mean number for functioning ovules becomes 15.3, which is just slightly above the expected mean for a 30-chromosome plant. The distribution of these plants among chromosome groups is approximately random.

The mean chromosome number of the 102 plants used to determine the chromosome number of the functioning pollen of F_1 plants is 29.8. If 10 is subtracted, the mean number for functioning pollen becomes 19.7, a number far above the expected mean. The distribution is also very limited, with a range of but 4 chromosomes.

Three plants produced by using F_1 pollen on *Euchlaena perennis* have a mean chromosome number of 39.66, thus showing that the gametes of the F_1 that functioned in their production had 19.66 chromosomes, a number that agrees very closely with 19.8 obtained from the larger population.

It is apparent that the chromosome number of the functioning ovules is in agreement with the chromosome numbers observed going to form the daughter nuclei at the first meiotic divisions. In the pollen, however, there is a marked discrepancy between the observed chromosome distribution at the time the pollen is formed and the chromosome numbers found in functioning pollen. The mean chromosome number, 19.8, shows clearly that there is a tendency for functioning pollen to be restricted to gametes with 20 chromosomes.

In the F_2 progeny the chromosome number ranges from 25 to 42, with a mean number of about 36. This is approximately the mean number expected from combining the mean numbers for functioning ovules and pollen. The distribution approaches that expected from combining the distribution of the ovules and pollen. Three plants, however, with the chromosome numbers 25, 26, and 27, respectively, seem to be sufficiently segregated from the major group to suggest that these three plants were produced from pollen with approximately 10 instead of 20 chromosomes.

TABLE 3.—*Chromosomes in back crosses on Zea mays and selfed back crosses*

Female parent		Male parent		Number of plants with indicated chromosome number																					Total plants	Mean chromosome number		
Species or back cross	Chromosomes	Species or back cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		Observed	Expected	Of functioning gametes of hybrid parent
	No.		*No.*																						*No.*			
Z. mays × (*E. perennis* × *Z. mays*)	30	*Z. mays*	20	8	13	20	32	25	16	10	4	1	2		1										132	23.44	25	13.44
Z. mays	20	*Z. mays* × (*E. perennis* × *Z. mays*).	30	6	1	1				1	2	3	14	13	5	1									47	27.87	25	17.87
Z. mays × (*E. perennis* × *Z. mays*).	30	do	30	4	1	5	3	3	4	2	5	1	5	12	6	14	22	28	25	17	8	10		4	179	32.44	30	

Table 3 presents the data from back-crossed plants that have been back-crossed again onto maize or selfed. The hybrid parents given in this table have 30 chromosomes, and so do not differ in chromosome number from the F_1 plants given in table 2. The chromosome complement has been appreciably changed, however, for instead of the 30 chromosomes being 20 teosinte and 10 maize, as is found in F_1 plants, there will be approximately 10 teosinte and 20 maize chromosomes if autosyndesis has been general.

The mean chromosome number of the functioning ovules of these back-crossed plants (having two sets of maize chromosomes in their chromosome complement), as measured by the chromosome number in plants from back crosses crossed with maize, is 13.44. This mean number is appreciably less than that found for the functioning ovules of F_1 plants and suggests that there is a tendency in this group of hybrids with a predominance of maize chromosomes to develop those ovules with chromosome numbers approximating the haploid chromosome number of maize.

The mean chromosome number of the functioning pollen of 30-chromosome back-crossed plants is 17.87. This number is also appreciably less than that found for F_1 plants. The more striking difference, however, is the bimodal distribution shown by these 47 plants, a distribution that was absent in the 102 plants used to measure the chromosome number of F_1 pollen, although suggested in the discussion of the slight bimodal tendency observed in the chromosome number of F_2 plants. The grouping of the chromosome numbers with one mode at 20.25 and another at 29.2 seems to demonstrate that functioning pollen tends to approach very closely the haploid chromosome numbers of maize or teosinte.

The mean chromosome number of selfed 30-chromosome back-crossed plants is 32.44 which is a little above the sum of the means of the chromosome numbers of the functioning ovules and pollen of back-crossed plants. The difference, however, is not significant, and the distribution of the plants among the various chromosome classes, although too complicated for a complete analysis, suggests two modes—one a little below 25 and the other a little below 35.

Table 4 is made up of additional data for various hybrids. It is apparent that in those progenies in which the female ancestor is the hybrid parent and the male is maize the chromosome numbers of functioning ovules of the female ancestor tend to approach the basic number, 10; but this tendency, although apparent, is sufficiently flexible to allow ovules with any chromosome number between 10 and 20 to function. Some additional data are presented to show the chromosome numbers of pollen of several complicated hybrids. The male ancestors of these progenies, largely maize in their composition, are found to have, in functioning pollen, chromosome numbers approximating the basic number, 10.

In no plant used to show the chromosome numbers of functioning pollen has a gamete been found having 23, 24, or 25 chromosomes—numbers that in many progenies would be expected to predominate. This is a clear indication that the chromosome numbers in functioning pollen are modifications of the numbers that are prevalent in the gametes at the time of their formation. Such modifications apparently result from a differential death rate during development or from a differential growth rate down the pollen tubes, or from both.

TABLE 4.—*Chromosomes in various Euchlaena perennis-Zea mays hybrids*

Female parent		Male parent		Number of plants with indicated chromosome number																				Mean chromosome number		
Species or cross	Chromosomes	Species or cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	Plants	Actual	Expected	Of functioning gametes of hybrid parent
	No		No																				No			
Z. mays × (*E. perennis* × *Z mays*)	29	*Z mays*	20	1		3	2		1			1											8	23.12	24.5	13.12
Z. mays	20	*Z mays* × (*E perennis* × *Z mays*)	31	6	1	1					1	1	10	2									22	25.82	25.5	15.82
Z mays × (*E. perennis* × *Z. mays*)	28	do	28		1	1									2	4	2						10	29.90	28	......
Do	29	do	29		1	4	3	6	1	2					1	1	2	3	2				26	26.92	29	......
Do	31	do	31		1	1		1				1		1	1	1	1	3	2	4	2		19	32.16	31	......
Z. mays × (*E perennis* × *Z mays*) S P [a]	27	*Z mays*	20	5	7	7	9	3	2	2													35	22.34	23.5	12.34
Do	33	do	20			3	11	22	29	26	5	2	1										99	24.93	26.5	14.93
Z. mays	20	*Z mays* × (*E perennis* × *Z mays*) S P [a]	27	70	14	1																	85	20.19	23.5	10.19
(*Z mays* × (*E perennis* × *Z mays*) × *Z mays*	22	*Z mays*	20	25	25	10																	60	20.75	21	10.75
Z. mays	20	(*Z mays* × (*E perennis* × *Z mays*)) × *Z. mays*	22	60	1																		61	20.02	21	10.02
Z. mays × (*Z. mays* × (*E. perennis* × *Z mays*))	29	*Z mays* × (*Z mays* × (*E perennis* × *Z mays*))	29	1	2	4	3	2	2	2						1		1					18	24.17	29	......
Do	31	do	31					1	1					2	1	2	3	1	2	2	1	1	17	32.59	31	......
Z mays (F_2)	29	*Z mays*	20		4	17	10	14	12	5	1												63	23.51	24.5	13.51
Do	31	do	20			6	8		7	2	1												24	23.75	25.5	13.75
Z mays	20	*Z mays* (F_2)	29	22	3																		25	20.12	24.5	10.12

[a] Selfed progeny

The data presented in table 4 to show the chromosome numbers prevalent in selfed progenies do not seem to merit extensive analysis, but they do indicate a bimodal tendency that must be due in large measure to the differential functioning of pollen having the basic chromosome numbers of either the maize or the teosinte parents.

DISCUSSION

The foregoing data indicate that in hybrids between *Euchlaena perennis* and *Zea mays* pollen effective in fertilization has a chromosome number approaching the chromosome number of the pollen of either of the ancestral forms, i.e., 10 or 20. In F_1 plants, as measured in back crosses, there is a very marked tendency for pollen with 20 chromosomes to effect fertilization. However, three F_2 plants with low chromosome numbers suggest that pollen with about 10 chromosomes has occasionally functioned.

That pollen grains with 10 chromosomes do, in fact, occasionally function is substantiated by the chromosome counts made in back crosses of various hybrids on corn. The functioning pollen from most back crosses on corn has approximately 20 chromosomes, but one progeny included in the data showed that pollen effective in fertilization approached the two extremes, 10 and 20, in about equal numbers. The pollen from the more cornlike hybrids derived from crossing a back cross onto corn had invariably 10 or approximately 10 chromosomes.

All studies made of many different types of hybrids between teosinte and maize have shown a complete absence of pollen with 13, 14, and 15 chromosomes. The high percentage of empty pollen grains characteristic of the F_1 plants indicates that there has been an elimination of pollen through death early in its development. In other hybrids, however, there is conclusive proof that after pollination there is a differential death or growth rate which eliminates pollen other than that having approximately 10 and 20 chromosomes. This is shown by contrasting the percentage of waxy pollen in mature pollen and the percentage of waxy seeds obtained when this pollen is used to pollinate homozygous waxy plants.

The data accumulated to determine the number of chromosomes in functioning ovules tend to show that ovules are distributed at random among the various classes. The mean chromosome number observed is frequently not a significant departure from the calculated mean. There are, however, in some of the more cornlike progenies significant departures that show a tendency for the selection of gametes with low chromosome numbers. It seems, therefore, that in the ovules there is a slight tendency to favor the survival of the parental chromosome numbers, but the data are too meager to show a bimodal distribution even if it was present. It is evident, however, that the differential survival of gametes possessing parental numbers is much less marked in the ovules than in the pollen.

The effect of the pronounced tendency for euploid gametes to be more virile—a tendency very pronounced in the pollen and suggested in the ovules of teosinte-maize hybrids—is to produce many plants with 20 and 40 chromosomes.

The chromosome complement, however, in these 20- or 40-chromosome plants is profoundly affected by the prevalence of autosyndesis. A hybrid with 2 sets of teosinte chromosomes will produce gametes with 1 set of teosinte chromosomes irrespective of the number of maize chromosomes present, or if the 2 sets of chromosomes are from maize there will be 1 set of maize chromosomes in all gametes formed. Such a chromosome distribution leads to the presence of 2 sets of teosinte chromosomes in all F_2 plants and 2 sets of maize chromosomes in all plants of selfed progenies from back crosses on maize.

The number of maize chromosomes in F_2 progenies and in back crosses on maize and the number of teosinte chromosomes in selfed progenies of these back crosses depend upon the absence of complete autosyndesis and upon the utilization of gametes with aneuploid or with the diploid chromosome numbers.

Cytologists have frequently reported that in hybrids between parents differing in chromosome number there occurs a differential survival of gametes bearing the parental number of chromosomes.

Täckholm (*21*), in his study of polyploid roses, showed that in the *canina* roses there is a marked tendency to mature pollen with a chromosome number near the basic number, 7. He has shown, on the other hand, that this class of roses makes ovules with only 7 chromosomes less than the somatic number of the plant under investigation. Blackburn and Harrison (*2*) have shown that in a pentaploid rose the functioning pollen has 7 chromosomes and that the egg cells have 28.

The elimination of all pollen except that with 7 chromosomes is in harmony with the selection found in many progenies of *Euchlaena-Zea* hybrids in which only those pollen grains functioned that had the basic chromosome number 10 or approximately 10.

The studies of the distribution of chromosomes in wheat hybrids and the number of chromosomes in functioning gametes is touched upon by Sax (*18*). In a later paper (*19*) he goes more fully into the chromosome number of functioning gametes of a 35-chromosome hybrid and finds that both male and female gametes have 14 chromosomes more frequently than would be expected on the assumption of a random distribution of 7 single chromosomes. Thompson (*22*), and more recently Thompson and Cameron (*23*), find that in a similar wheat cross there is an elimination of gametes with chromosome numbers between the two modes 14 and 21, which is more pronounced in the pollen but significant in the ovules. Watkins (*24*) finds that in a pentaploid wheat hybrid the ovules are generally fertile, whereas in the pollen grains there is a high degree of sterility. He finds further that pollen grains possessing either 14 or 21 chromosomes are more likely to function than those having other numbers of chromosomes.

The random distribution of unpaired chromosomes of wheat hybrids at the time the gametes are formed and the later elimination of gametes with chromosome numbers between the two extremes, as described by the foregoing investigators, are very similar to the conditions found in this study of *Euchlaena-Zea* hybrids. In these hybrids gametes with all chromosome numbers between the two modes are formed, but in the pollen and to a lesser degree in the ovules there is a marked tendency to eliminate all but the low and the high chromosome numbers.

The studies by Goodspeed, Clausen, and Chipman (*10*) and by Goodspeed and Clausen (*9*) of *Nicotiana* hybrids show in some cases that a triploid distributes its 12 univalent chromosomes at random in the first division and that there is a random survival of pollen in the different chromosome classes at the time of fertilization.

Karpechenko (*12*) has found that viable gametes of F_1 hybrids of *Raphanus* × *Brassica* ($2n=18$ chromosomes), usually have 18 chromosomes—9 *Raphanus* and 9 *Brassica*. The pollen of F_1 *Euchlaena-Zea* hybrids is similar in constitution to that described by Karpechenko, since in both cases there is reason to believe that the chromosome complement of most of the functioning pollen is made up of one set from each of the parents.

Blakeslee and Farnham (*3*) found that daturas having $2n+1$ chromosomes produce ovules with n, $n+1$, and $n+2$ chromosomes, whereas practically no pollen that functions carries extra chromosomes. McClintock (*15*) has shown that in *Zea* plants which have extra chromosomes there is a decided selection against extra chromosomes in the male gametes and a less obvious selection against eggs carrying extra chromosomes.

It is apparent that selection of gametes with chromosome numbers approaching the basic number or a multiple of the basic number present in the hybrid's ancestors has occurred in widely separated groups of plants. In some cases the gametes have borne the haploid number of chromosomes and in others the diploid number. In most cases it seems that the gametes formed represent all chromosome numbers between the two extremes and that the selection takes place either by differential viability or by differential growth rate. One case, namely, that of the roses, has been described where the formation of ovules is irregular and the eggs lack only 7 chromosomes of the somatic chromosome number characteristic of the hybrid.

Where the chromosome complement of a plant is made up of chromosomes in addition to the two homologous sets, the tendency of the functioning gametes to have the basic chromosome number or a multiple of this number must lead to the production of plants with chromosome numbers in multiples of the basic number and to the absence of plants with aneuploid chromosome numbers.

Indirect demonstration of the selection of gametes is seen in the chromosome numbers of a plant group such as *Rubus* (*8*) or other similar groups which are found to have chromosome numbers only in multiples of the basic number and in which hybridization frequently occurs between forms with different chromosome numbers. The lack of aneuploid forms in such groups seems to be the result of gametic selection favoring the parental numbers.

If triploid hybrids utilized only euploid gametes, three groups of plants, namely, diploid, triploid, and tetraploid, would be found in the offspring.

The tetraploid plants represent a fairly stable group distinct from either parent and contain two sets of chromosomes of the two parents if autosyndesis has been the rule. Darlington (*5*) has stressed the value of such hybrids in productive plant breeding. It is true that in tetraploids there is a proportionate increase in genes with an increase in chromosomes, but a crypt hybrid may breed true for many generations with only rare indications of its hybrid nature. If autosyndesis prevails, a tetraploid may defy our best efforts to segregate and stabilize hidden genetic characters.

Gigantism has frequently been associated with marked increase in chromosome numbers, and consequently tetraploids should be desirable in cases where gigantism is a desired factor. Tetraploid perennial teosinte, however, is no larger than the diploid annual form.

Gregory (*11*) and Sinotô (*20*) have demonstrated that gigantism occurs in forms having few chromosomes as well as in forms having many. Frost (*7*) and Longley (*14*) have found that tetraploid citrus plants are dwarfs as compared with their diploid relatives.

It seems to have been rather generally accepted that in isolating improved strains from polyploid hybrids the progenies with a large number of chromosomes provide the most desirable material. The idea may have originated from the cases of gigantism associated with a doubling of the chromosome number or from the fact that improved varieties tend to have a higher chromosome number than unimproved or wild stocks.

It has been pointed out that only a small percentage of tetraploid forms exhibit gigantism. The two tetraploids produced in the present study showed no increased vigor. The increased number of chromosomes in improved varieties may be accounted for in another way. Chromosome number is a character which was not considered in the breeding of existing varieties but which has been observed since the varieties were developed. If favorable variations occurred in the same ratio in diploid and tetraploid derivatives but if tetraploid derivatives were more numerous, high chromosome numbers would predominate in the varieties finally produced. In the descendants of the maize-perennial teosinte hybrids the mean chromosome number is well above the mean of the parents, but no correlation between chromosome number and vigor could be detected.

The final question to consider is that of the bearing of chromosome number on the recombination of parental characters and the ease with which desired combinations can be stabilized.

In triploid hybrids where only euploid gametes function, the isolation and stabilization of a new combination of genes is possible only when allosyndesis occurs in the pairing of the homologous chromosomes. If autosyndesis always occurs, the tetraploid segregates will combine the parental characters in heterozygous forms and these forms will reproduce themselves as long as autosyndesis continues to prevail, but no new homozygous combination of genes is possible. When allosyndesis occurs, three groups of segregates, namely, diploid, triploid, and tetraploid, are obtained, all of which combine the ancestral characters, and from any form containing the desired combination homozygous plants can eventually be isolated.

Because of the formation of both haploid and diploid gametes, the triploid segregates are too complex to be discussed here, but the relative advantage of high and low chromosome numbers may be estimated from diploid and tetraploid derivatives. Assuming that the triploid hybrid is heterozygous for two simple characters located in different chromosomes and that is is desired to fix a nonparental combination of the characters, it follows that:

(1) In the haploid gametes the four possible combinations of dominants and recessives will be represented in equal numbers, and in selfed F_2 individuals 1 out of 16 will be homozygous for any desired combination.

(2) In the diploid gametes there will also be four classes, but none of them will be pure for nonparental combinations. Three quarters or nine sixteenths of the selfed F_2 individuals will carry the desired com-

bination but they will not be homozygous; it will therefore be necessary to grow a third or even a fourth generation before the combination can be obtained in a homozygous form.

Consequently it is apparent that a diploid segregate supplies all the parental combinations that may be found in a tetraploid segregate and offers a distinct advantage when an attempt is made to isolate and stabilize any particular combination.

The behavior of F_1 teosinte-maize hybrids has shown that it is possible to obtain from a tetraploid form a diploid form having a chromosome complement made up of 2 of the 4 sets of the tetraploid. From selfing back crosses on maize, plants may be obtained having chromosome complements made up entirely from the chromosomes of the diploid parent.

These findings suggest the possibility that diploid maize may have originated from a hybrid between tetraploid perennial teosinte and some unknown diploid relative. It is much simpler, however, to assume that this hybridization occurred between two diploid forms whose blended characters produced the ancestor to maize, and that *Euchlaena perennis* perhaps does not represent a remnant of a once-prevalent form but rather a recent tetraploid form of the widely distributed annual teosinte.

In the foregoing general discussion of gametic selection in hybrids whose ancestors differed in chromosome number, as well as in the particular discussion of gametic selection in *Euchlaena-Zea* hybrids, the point is emphasized that the selective survival of gametes will profoundly affect the nature of the forms recovered in later generations. The illustrations are not perfect examples of the survival of gametes possessing the basic chromosome number (or multiples of this number) of the form involved. If this tendency were perfect (and it does not seem unreasonable to assume that in many cases it is) its effect on future offspring would be apparent. It seems probable that in nature this differential survival has produced as many recombinations with low as with high chromosome numbers. In this connection the occasional appearance of tetraploids must be cardinal, forming part of an evolutionary process that tends to keep the chromosome number in plant groups of recent origin no higher than those found in the older groups.

SUMMARY

The meiotic behavior of the chromosomes in various teosinte-maize hybrids indicates that homologous chromosomes usually pair and that the unpaired univalent chromosomes are distributed at random to the daughter nuclei. The division of these univalent chromosomes occurs in either the first or the second division.

The regular distribution of all paired chromosomes and the random distribution of all unpaired chromosomes result in gametes with various chromosome numbers ranging from the number of pairs to the number of pairs plus the number of unpaired chromosomes.

The chromosome number of functioning pollen of the various teosinte-maize hybrids is found to approximate either 10 or 20, the haploid chromosome numbers of the two parents, while the chromosome number of functioning ovules shows only a slight tendency toward the euploid numbers, 10 and 20.

An increased viability of euploid gametes seems to be general in hybrids similar to the teosinte-maize hybrids here described, and the survival of gametes with these chromosome numbers profoundly affects the character of the progeny of hybrids.

It is suggested that the diploid derivatives of hybrids offer favorable experimental material. If allosyndesis has occurred, even the gametes with the haploid chromsome number of the diploid parent will contain chromosomes and consequently characters from both parents. A diploid derivative will, therefore, combine the ancestral characters of the parents in a plant that seems just as promising for breeding material as those forms with twice as many chromosomes.

The utilization of euploid gametes from tetraploid hybrids restricts the plants of their progenies to three chromosome groups; namely, diploid, triploid, and tetraploid. The prevalence of diploid forms combining the characters of the ancestors suggests that gametic selection has led to the production of new and eventually stable forms without increasing the chromosome number above that of the diploid parent.

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A BACTERIAL DISEASE OF HEDERA HELIX

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INTRODUCTION

In the spring of 1930 a shipment of English ivy, *Hedera helix* L., was received in New Jersey from Maryland, heavily infected with a bacterial leaf-spot disease. These plants were grown outdoors during the summer, and infections failed to appear on the new growth. Several thousand cuttings were taken from them. In the fall, when these rooted cuttings were potted and grown under greenhouse conditions, the disease reappeared in epidemic form, killing many plants and rendering the remainder worthless. Since a bacterial leaf-spot disease of this host had not previously been mentioned as occurring in the United States, and since under certain conditions it causes serious losses, investigations of its cause and nature were undertaken. The results are reported in this paper.

LITERATURE REVIEW

Lindau (*5*)[2] in 1894 described a bacterial leaf-spot and stem-canker disease of English ivy in Germany. From his description and illustrations there is no doubt as to the identity of our material and his. No inoculation tests were attempted by Lindau. In his cytological work he failed to find evidence of stomatal infection and concluded that infection took place through the natural wounds on the stem caused by the sloughing off of the pubescence normally present on the tips of young growing stems. Rapidly growing plants were noted as being more susceptible than slower growing plants.

Arnaud (*1*) in 1920 redescribed the disease from France, giving the name *Bacterium hederae* to the organism he isolated. Although he failed to describe the organism or to report any inoculation trials to prove its pathogenicity, his description of the symptoms again leaves little doubt as to the identity of the disease.

Killian (*3*) in 1921 reported successful inoculations with *Bacterium hederae* Arnaud, thus proving the pathogenicity of the associated organism. He also described gross characteristics of the organism on several media. The incubation period was determined as from 1 to 3 weeks, depending on the temperature and humidity. He failed to obtain infection on old plant parts, even where wounded.

The first mention of this disease in the United States was made by White[3] in December 1930, followed by rather complete descriptions in August 1931 (*8*).[4]

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[1] Received for publication Dec 1, 1933, issued July 1934 Cooperative investigations between the New Jersey Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture. Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology

[2] Reference is made by number (italic) to Literature Cited, p 815.

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Burkholder and Guterman (*2*) have recently described the same trouble on plants shipped from Georgia to New York and have reported synergism as existing between *Bacterium hederae* and an associated organism also isolated from diseased areas.

ECONOMIC IMPORTANCE

The ivy disease described here has been reported from two commercial nurseries in New York (*2*) and New Jersey, respectively, into which it was imported on plants purchased from growers located in Georgia and Maryland. In 1933 it was found on outdoor-grown ivy in Virginia and in the District of Columbia. Its geographic distribution and importance in the Southern States has not been investigated. Burkholder and Guterman (*2*) give no data on its seriousness in New York.

In New Jersey it immediately became a serious pest in the one large commercial greenhouse into which it was introduced in 1930. Cuttings taken from the stock plants were seriously infected. Over 40,000 were either killed outright or so severely infected that they were discarded as useless. Infections on plants trained into pyramids rendered them unsalable. Under greenhouse conditions where ivy is syringed periodically the spread of the disease is rapid, owing to the dissemination of the bacteria from infected leaf areas and stem cankers by the water. Introduced on *Hedera helix*, this disease has spread to several of its horticultural varieties being grown by this same concern and has persisted and caused injury and losses in spite of all efforts to check it.

SYMPTOMS

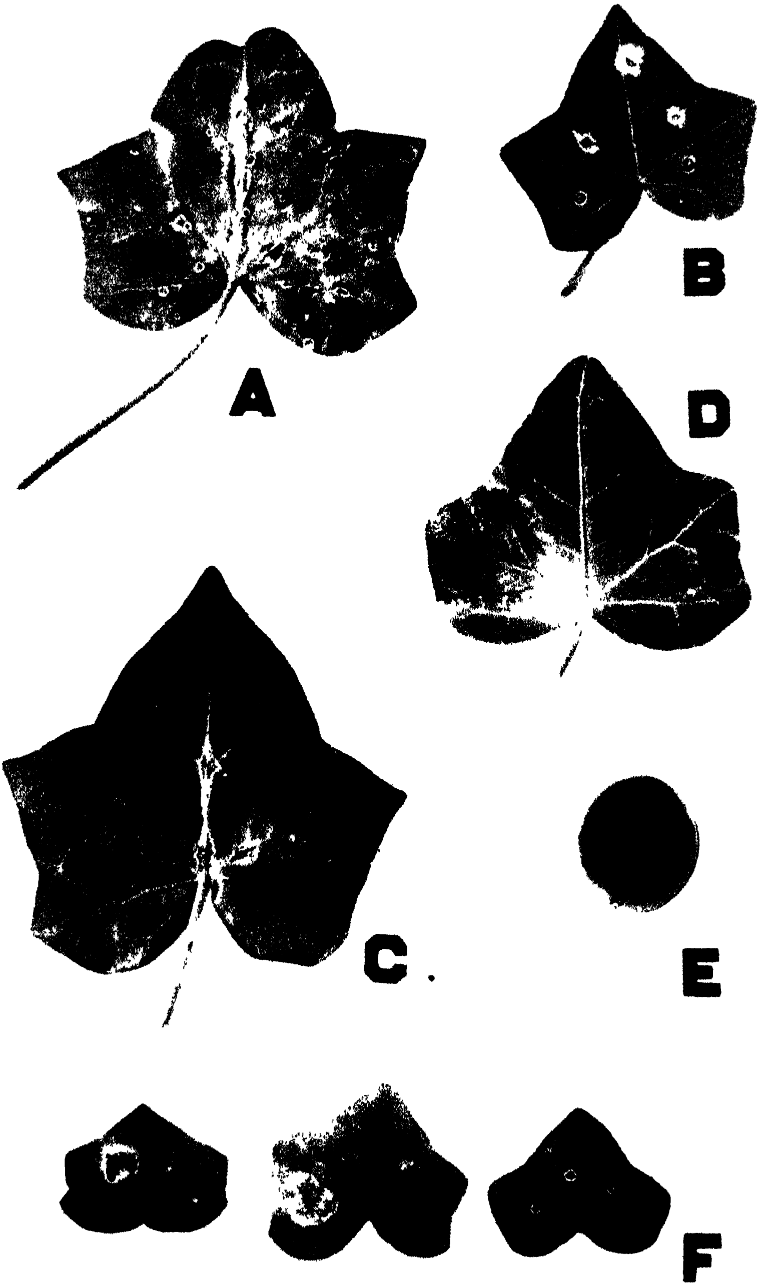
ON LEAVES

Recent infections on young leaves become evident in from 5 to 12 days as small translucent, roughly circular spots (pl. 1, *B*). In the earliest stages these spots are very difficult to see except by transmitted light. As the spots enlarge, the center becomes brown to brownish black, dries out, and frequently cracks (pls. 1, *A*, and 2, *A*). Under conditions of high humidity an orange-red bacterial exudate may occur on the infected areas. The older spots are usually surrounded by a light yellowish-green water-soaked area, but on old foliage this may be replaced by a reddish to reddish-brown irregular or scalloped region. Infected areas on the leaves are frequently secondarily infected with either *Colletotrichum trichellum* (Fr.) Duke (pl. 1, *D*) or *Phyllosticta hedericola* Dur. and Mont. This succession of *P. hedericola* following bacterial infections has previously been reported by Nicolas and Aggery (*6*) on *Aralia japonica*.

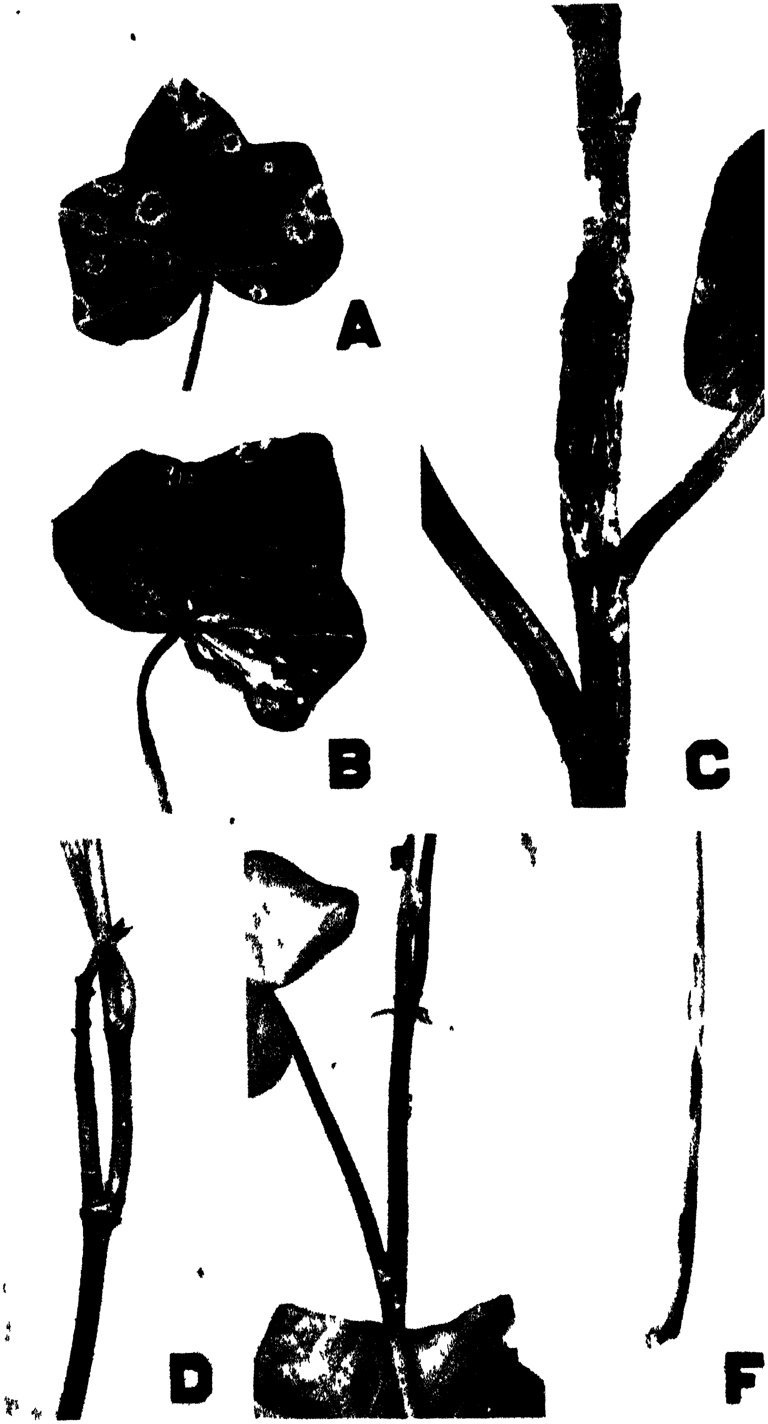
Infection frequently takes place on or very close to a vein. Under such conditions the spots developing are not circular but are elongated in the direction of the vein, indicating possible systematic invasion (pl. 1, *C*). Heavily infected leaves usually turn yellow and fall.

ON PETIOLES

Direct infection of petioles is rare. The spots that develop from such infections are dark brown to black and enlarge rapidly in both directions and soon girdle the petiole, and the attached leaf wilts (pl. 2, *B*, *F*). Petioles frequently become infected from heavily



A, Artificial inoculations obtained by brushing bacterial suspension on leaf with camel's-hair brush, B, needle-prick inoculations on young leaf after 8 days, C, artificial inoculations obtained by needle pricks in veins, D, leaf with four natural infections with *Bacterium hederae*, one of which has been followed by *Colletotrichum trichellum*, E, colony of *Bact. hederae* on beef-extract agar, F, leaf on left inoculated with *Bact. hederae*, center, double inoculation with *Bact. hederae* and culture *d*, right, double inoculation with *Bact. hederae* and culture *k*, all 4 weeks after inoculation



A, Natural leaf infections; B, natural leaf infections and petiole lesions, C, stem canker, D, infection at growing tip, E, bacterial exudate from very young stem canker, F, petiole lesions inoculated by bacterial suspensions brushed on petiole with camel's-hair brush.

infected leaves, however, the bacteria advancing rapidly down the petioles to the stem.

ON STEMS

Infection on stems takes place naturally either from infected petioles or on the very young and tender growing tip (pl. 2, *D*). On young tissues a soft dark-brown to black decay rapidly takes place. Invasion is retarded when older tissue is reached, a new growing point is developed from the next lowest axillary bud, and no further advance is made.

On older tissues of the stem, where infection arises from petioles, definite cankers are produced. At first these cankers appear as small brown sunken areas. Invasion of tissue is slow. Old cankers are flattened and shrunken, usually cracked longitudinally, and surrounded by swollen margins due to callus formation on the part of the host (pl. 2, *C*). Stem cankers on old tissues have never been observed entirely girdling the stem; however, they frequently cause a cessation or retardation of growth and an abnormal light-green coloration of the foliage. Frequently the foliage of plants carrying stem cankers develops a reddish-bronze coloration typical of that produced by maturity in the fall. An orange-red bacterial exudate frequently occurs on these stem cankers (pl. 2, *E*).

VARIETAL SUSCEPTIBILITY

Arnaud (*1*) noticed that of two ivies under observation, one, "Lierre des Bois", was more severely attacked than the other, "Lierre d'Ecosse." The disease has been observed occurring naturally on *Hedera helix* and its varieties *baltica*, *gracilis*, *lucida*, *digitata*, and Silver Queen. Inoculations upon these varieties as well as upon the varieties *marmorata*, *alba variegata*, *dentata variegata*, *conglomerata*, *nigra*, and *coriacea* have shown all to be susceptible.

PATHOGENICITY

The pathogenicity of the organism constantly associated with these disease symptoms has been repeatedly proved by inoculations in various parts of the host under varied conditions and by various methods. Repeated reisolations and reinoculations have been successfully made. Pure cultures are easily obtained by the usual poured-plate method. Young leaf tissues (plants kept under bell jars in a constantly moist atmosphere) are readily infected by atomizing with bacterial suspensions and show positive results in as short a time as 4 days. The tiny translucent spots appeared in 5 to 6 days, and some were 7 to 8 mm in diameter in 9 days. These lesions were in all respects similar to those found on naturally infected plants. Mature leaves or woody stems show symptoms only after longer periods, varying from 2 to 3 weeks. Such tissues are rarely infected except through wounds.

THE PATHOGENE

The cultures used for the morphological, cultural, and physiological studies were isolated from characteristic leaf lesions. Some of the lesions were the result of natural infections; others were the result of artificial inoculation with the bacteria. The pathogenicity of the several cultures used in these studies was established by successful infections induced by inoculation of healthy, growing ivy leaves and stems.

MORPHOLOGY

Bacterium hederae is a short rod with rounded ends, rather smaller than is usual for plant pathogenes. In culture media the rods are 0.7 to 2.7μ long by 0.3 to 0.6μ wide and occur singly or in pairs or short chains. In the host tissues they are 0.7 to 2μ long by 0.2 to 0.4μ wide. They are motile by means of one polar flagellum. Capsules are present. No spores have been found.

STAINING REACTIONS

The organism is Gram-negative; it is not acid-fast. It stains readily with all the commonly used bacteriological stains. The capsules are easily demonstrated with Ribbert's dahlia capsule stain and also with Leifson's (*4*) stain, a flagella stain which stained the capsules but only rarely the flagella of the ivy bacteria which proved to be unusually difficult to stain. However, with Casares-Gil's stain it was definitely determined that there is a single polar flagellum, usually long and often quite wavy in the part nearest the rod.

Carbol-fuchsin-stained mounts from potato-dextrose agar cultures grown at 34° C. showed single rods and chains very poorly and irregularly stained, with diameters varying from 0.3 to 0.9μ. In beef-media cultures the diameter of the bacteria is slightly greater than in potato-dextrose agar cultures.

CULTURAL CHARACTERS[5]

BEEF-PEPTONE AGAR COLONIES.—On beef-peptone agar (pH 6.8 to 7.0) the colonies of *Bacterium hederae* grow slowly. In 48 hours they are usually visible as mere points of growth, and in 4 to 5 days even well-isolated colonies are only 1 to 2 mm in diameter. In 10 to 12 days a few colonies are 5 to 7 mm in diameter, but the usual size is 2 to 4 mm. White and transparent at first, they become pale yellow, Massicot yellow,[6] and translucent. They are circular, smooth, glistening, slightly elevated, with entire margins (pl. 1, *E*). The interior has definite short concentric lines or crosshatching, which disappears when the colonies are 10 to 12 days old. Beef is not a very favorable medium for this organism, and the colony characters vary with even slight differences in the media, the acidity, the moisture, the temperature, or other factors. In one set of plates the colonies had definite white halos. In another set from 3 to 20 points of secondary growth appeared within each colony. Sometimes the centers are opaque with translucent crosshatched borders, or they may be granular, mottled, or homogeneous. Buried colonies are spherical to lenticular, opaque to translucent. The growth is slightly viscid. Another fact noted is that growth in transfers from beef-media cultures is very uncertain. Unless the beef culture is young and rather heavy inoculations are made from it, more often than not no growth develops in the transfers.

BEEF-PEPTONE AGAR SLANTS.—Growth is slow and never becomes even moderately heavy. If the inoculation is from a liquid culture the resulting growth is most likely to be in the form of tiny isolated colonies, which eventually may coalesce. Inoculation from agar cultures gives a uniform, smooth, glistening streak, practically colorless except on the lower part of the slant, where the growth is somewhat thicker and pale yellow. Crosshatching or striae are present. The growth is slightly viscid or elastic; it does not draw out in a long thread but breaks at a length of 4 to 6 mm.

BEEF-EXTRACT AGAR.—Growth is very similar to that in beef-infusion agar, but the color is slightly deeper yellow and the growth is not viscid.

BEEF-PEPTONE BROTH.—The bacteria grow slowly in beef broth, even of most favorable pH value and at favorable temperatures. Thin, rolling clouds, best in the upper layers of the liquid, appear in 2 days. Growth increases slowly for a number of days, but the clouding is never more than moderate. Irregular rims of pale yellow appear in 5 to 7 days and often become fairly wide and heavy. If cultures are undisturbed a thin pellicle forms. There is a moderate, translucent sediment, which rises in a spiral when shaken. Rims, pellicles, and sediment are viscid. Clouding persists for 5 to 6 months at room temperature.

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[5] Unless otherwise stated, all beef media were made with beef infusion and had a pH of 6.8 to 7.0. Cultures were grown at about 23° C.

[6] RIDGWAY, R COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

Potato-dextrose broth.—In this medium, with a pH value of 5.6 to 5.8 the liquid clouds very slightly, but the surface growth in the form of rim and pellicle is heavy.

Potato-dextrose agar.—This medium with a pH value of 5.6 to 5.8 seems to be ideal for the ivy bacterium. In isolation plates the colonies reach a diameter of 10 to 12 mm. In tubes of slanted medium the surface is quickly covered with a thick, smooth, glistening layer of homogeneous to indefinitely mottled (under a × 6 lens) growth. The growth, practically colorless at first, becomes pale greenish yellow, Massicot yellow, or Chartreuse, and later chamois [7] or even darker. The texture of the growth is soft and butyrous. Old cultures sometimes show a trace of viscidity. The thick, smooth, translucent growth remains practically unchanged for weeks. The pH of the medium of cultures changes in 3 days from 5.6 to 6.6.

Potato cylinders.—On steamed potato cylinders growth at first is fairly smooth and pale yellow, but it soon becomes thin, wet, and yellowish brown with some pale yellow at the margin. The potato is moderately browned. Growth does not persist or increase. A weak diastatic action was indicated by tests with Lugol's iodine solution.

Beef gelatin.—In plates of beef gelatin with a pH of 7.3, the colonies were barely visible in 2 days at 20° to 23° C. In 5 days the gelatin immediately below the colonies was liquefied. In 7 days the medium of thickly sown plates was entirely liquefied and the small, spherical, compact colonies were floating in the unclouded liquid. In tube cultures slight liquefaction is evident in 2 days. In 6 to 7 days there is a 5- to 7-mm stratiform layer of liquid. Further advance is slower, 5 to 6 weeks being required for liquefaction of the entire 10 cc of medium. The liquefied gelatin is almost entirely clear. Irregular rims and thin pellicles of pale yellow form, and there is a moderate viscid sediment, deeper yellow and more opaque than in beef-broth cultures.

Blood serum.—On Loeffler's solidified blood serum, growth was doubtful for several days. Later the whole slant was covered with a smooth layer of mustard-yellow growth. No trace of liquefaction was observed until after 4 weeks, when the slanted part became translucent and yellowish. In 7 weeks some cultures were entirely liquefied, while in others only the slanted portion was liquefied. Cultures were now getting dry, and no further change occurred.

Reduction of nitrates.—Growth is fairly good in nitrate-beef bouillon, but 9-day-old cultures with the starch-iodine-sulphuric acid test showed no trace of nitrate reduction. Cultures in a synthetic nitrate medium tested when 10 days old with the α-naphthylamine sulphanilic-acid as recommended in the Manual of Methods (*7*), gave positive indications of a moderate reduction of nitrate, less than half as much as in control cultures of *Bacillus phytophthorus* and *B. aroideae*.

Diastatic action.—Plates of beef agar plus 0.2 percent of starch were heavily inoculated with surface streaks. Growth was not vigorous. On the eighth and tenth days a partially cleared zone 15 to 20 mm wide appeared when the plates were flooded with iodine solution. Potato-cylinder cultures 5 weeks old tested with iodine also gave indication that the starch is only partially hydrolyzed.

Cohn's solution.—Repeated trials with light and with heavy inoculations show that the organism does not grow in this medium.

Uschinsky's solution.—When heavily inoculated a slight milky color and thin clouding appeared after 6 to 10 days. In 4 to 6 weeks the clouding was moderate and fairly heavy and pale yellow rims and pellicles formed. There was no color change in the medium.

Fermi's solution.—Growth is slow and slight in this medium. Heavily inoculated cultures 3 weeks old are faintly clouded and have a few slender white threads suspended in the liquid or attached to the tube wall. There is no rim of pellicle and no color change in the medium.

Toleration of sodium chloride.—In beef broth containing 1 percent of NaCl, growth is as good as in plain broth. Growth is slightly retarded by 2 percent, greatly retarded by 3 percent, and entirely lacking in 4 percent of NaCl.

Indol production.—Indol is not produced. Cultures in a 2-percent peptone solution and in 1-percent tryptophane solution grow better than in beef broth.

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[7] Ridgway, R. See footnote 6.

The tests were made with sulphuric acid and sodium nitrite. Control cultures of *Bacillus coli* produced indol.

HYDROGEN SULPHIDE PRODUCTION.—A slight amount of hydrogen sulphide is produced. Tests were made in lead acetate agar; also by strips of lead acetate paper suspended over cultures.

AMMONIA PRODUCTION.—Slight amounts of ammonia are produced in beef media and in peptone-broth cultures.

MILK.—Milk is slowly coagulated. The curd remains soft and jellylike for 2 to 3 weeks, then becomes more compact. Casein is slowly digested, about 3 months being required for complete digestion. The whey is yellowish and viscid. Numerous tyrosin crystals form in all milk cultures.

LITMUS REDUCTION.—Lavender-colored litmus-milk shows slight to no bluing. Reduction of the litmus begins in 4 to 8 days and is complete in 6 to 12 days.

METHYLENE BLUE REDUCTION.—Methylene blue in milk is considerably reduced in 2 and entirely reduced in 8 days.

FERMENTATION OF CARBOHYDRATES.—The ability of the bacteria to ferment carbohydrates was tested on peptone-free synthetic agar (7), with brom-cresol purple as an indicator. One percent each of dextrose, sucrose, lactose, maltose, mannite, and glycerin was used. Acid without gas was formed very promptly from dextrose and sucrose, rather slowly from lactose, and very slowly from glycerin. In maltose there was only a trace of growth and no acid reaction. There was no growth in mannite.

TEMPERATURE RELATIONS.—The optimum temperature for growth is between 20° and 26° C. Beef-bouillon cultures cloud more readily at 25° to 26° than at 20° to 22°, but after several days the cultures at the lower temperatures have the better growth. The minimum temperature for growth is 2° or lower. The maximum for beef-bouillon cultures is 32°. (Beef-bouillon cultures cloud thinly at 33° and 34° in 1 to 2 days, but this clouding disappears in 1 day or less.) Slight but persistent growth occurs on beef agar at 34° and on potato-dextrose agar at 35° and 36°. No growth occurred at 37°. The thermal death point is near 52°.

EFFECT OF FREEZING.—Potato-dextrose agar cultures held at 4° to 8° F. (−20° to −22° C.) for 4½ months, except for several short intervals of partial or complete thawing, were not killed or even noticeably reduced in vitality.

EFFECT OF SUNLIGHT.—Freshly inoculated plates of beef agar with half of each plate covered to exclude direct light were exposed for 10, 20, and 30 minutes to direct sunlight at midday, April 5, 1932. A slight haze but no clouds slightly reduced the light. In these tests the bacteria were killed in the areas subjected to the direct rays of the sun, and also to a considerable distance under the covered parts. Colony numbers were normal only in the area farthest from the light. In a repetition of the experiment at midday, April 12, 1932, when there was no haze, all bacteria were killed by exposure for 10 minutes; in 6 minutes 80 to 90 percent were killed, and in 3 minutes 40 to 60 percent were killed.

OPTIMUM REACTION AND TOLERATION LIMITS.—Because of the slow growth of the bacteria the pH values of the medium may change somewhat before the cultures cloud. Repeated tests, with the pH values of the media determined at inoculation time and again when growth became evident, indicate 7.0 as the optimum for growth, and 5.5 and 8.5 as the limits The different strains studied varied slightly, and at different times the same strain sometimes showed slight variations in the pH requirements.

RELATION TO OXYGEN.—The organism is aerobic. There was no clouding in the closed ends of fermentation tubes containing beef bouillon and synthetic media plus carbohydrates, nor in agar tubes either with or without carbohydrates.

VITALITY IN CULTURE.—In culture media, particularly in beef media, the vitality of the ivy bacterium shows a lack of uniformity. At room temperature some beef cultures have remained alive for 7 months, but most cultures die within 2 to 8 weeks. Many transfers to beef media, especially if made from liquid culture, fail to establish any growth. On potato-dextrose agar there is no difficulty in securing vigorous growth, and no reduction in vitality has been noted in cultures of various ages up to and including some 9 months old. At temperatures below freezing, the organism remains alive at least 4 months on potato-dextrose agar. Cultures that grew slightly at 34° and 35° C. and remained at these temperatures for 20 to 30 days were to some extent reduced in vitality, as shown by growth in transfers under favorable conditions but by failure to grow under slightly adverse conditions.

EFFECT OF DESICCATION.—Tests for resistance to drying were made by placing drops from beef-bouillon cultures or growth from agar cultures diluted in water or broth on cover glasses, and after these had dried, introducing them at intervals into culture media. As in transfers from beef cultures, there was a lack of uniformity in growth results. A large number of covers failed to give any growth after drying even a few days. Some, however, produced typical growth after drying for 43 days. If the covers were put into beef bouillon, growth very seldom developed. The best method found was to embed the cover partially in very moist potato-dextrose agar.

HISTOLOGY

Thin, stained sections of ivy leaves collected 9 days after inoculation by spraying with suspensions of *Bacterium hederae* show numerous small infected areas on the lower side of the leaf. Bacteria are abundant in these lesions, most of which have penetrated only a short distance; others extend through half the thickness of the leaf and spread laterally an equal distance. In these sections the lesions are advanced to a stage where the lower epidermal cells are broken or distorted, and cases of distinct stomatal infection have not been seen. Stomata are present on the lower surface only of the ivy, and since infection starts on the lower side of leaves inoculated by spraying, it seems more than probable that the infection is stomatal. The numerous and large intercellular spaces in these leaves afford good accommodations for masses of bacteria. First the bacteria occupy the intercellular spaces; later the cell walls break. In some cases the bacteria seem to be inside intact cell walls. In sections of older lesions there is considerable breaking down of cell walls, the bacteria occupy large pockets, and the upper as well as the lower epidermis is destroyed. Sections of leaves collected 48 hours after inoculation show no sign of infection. The bacteria in the lesions are 0.7 to 2μ by 0.2 to 0.4μ wide. Capsules were stained on rods direct from leaf lesions. Staining of flagella on leaf-lesion rods was not attempted.

TECHNICAL DESCRIPTION

Bacterium hederae Arnaud is a short, motile rod, 0.7 to 2.7μ by 0.3 to 0.6μ, with a single polar flagellum. Capsules are produced, but no spores. It is Gram-negative and is not acid-fast. It is aerobic. Gelatin and blood serum are slowly liquefied. Nitrate is slightly reduced, but no gas is produced. Diastatic action is slight. Acid without gas is formed from dextrose, sucrose, lactose, and glycerin. The organism does not form acid in milk, but reduces litmus and methylene blue in this medium. Indol is not formed. Slight amounts of hydrogen sulphide and ammonia are produced. Its optimum pH value for growth on beef media is 7.0, and the optimum temperature for growth is 20° to 26° C., maximum 36°, minimum below 2°. Its thermal death point is 52°. On beef agar growth is moderate to slight. Colonies are round, smooth, pale yellow. On potato-dextrose agar growth is abundant, pale yellow. It causes leaf spots and stem cankers on English ivy, *Hedera helix*, and its horticultural varieties.

SYNERGISM

With the appearance of Burkholder and Guterman's (*2*) report of synergism between *Bacterium hederae* and an associated organism, experiments were conducted with 10 associated bacteria which the present writers had isolated and designated as cultures *d* to *m*. Without exception these associated organisms were isolated from old spots on foliage. Isolations from young diseased areas never failed to yield pure cultures of *Bact. hederae*.

Inoculations were made on leaves injured by needle pricks with *Bacterium hederae* in pure culture and also mixed with each of the 10 associated bacteria. In one case when culture *d* was mixed with *Bact. hederae* a distinct increase in size of the infected area resulted, which was still evident after 4 weeks from the time of inoculation. In all other nine mixed inoculations (*Bact. hederae* plus cultures *e–m*), the exact reverse process took place, or a case of anergism. The spots resulting from these mixed inoculations after 1 month were approximately 2 mm in diameter and lacked the water-soaked margins characteristic of active invasion of tissue. Inoculations with *Bact. hederae* in pure culture produced spots 7 mm in diameter, and with *Bact. hederae* and culture *d* 12 mm in diameter in the same time (pl. 1, *F*).

Organisms *d* and *k* are not the associated organisms reported by Burkholder and Guterman (*2*). Their identity is unknown. The anergistic action of nine other associated organisms (*e* to *m*, inclusive) is worthy of note.

CONTROL MEASURES

As measures of control of this disease, all soil, sand, or cinders on which an infected lot of plants have stood should be removed and the beds or benches sterilized by washing or spraying with formaldehyde solution 1:50 or corrosive sublimate 1:1,000, before placing another lot of potted cuttings on them. If the infection on any lot of plants is slight, hand-picking of the infected foliage, the pruning out of all tip infections, and the discarding of plants showing stem cankers can be resorted to and will lessen the danger of increasing severity. Keeping the temperatures of the houses at 50° F. or below has also seemed to check the rapidity of spread. Excessive syringing of the plants should be avoided, as this practice tends to spread the disease, particularly from plant to plant in the same bench

The use of protective sprays on ivy is not desirable, because of the residue deposited by them on the foliage. Potassium permanganate has been tried in concentrations as high as 1:600. At this concentration some injury took place, but injury was absent at a concentration of 1:800. The grower felt that considerable advantage from the standpoint of disease control was obtained after repeated applications, although no unsprayed plants were held as checks against these sprays. Mercuric bichloride at a concentration of 1:1,000 has also been used, and this caused slight injury to the young foliage.

Preliminary tests were made with proprietary organic-mercury sprays containing as the toxic ingredient ethyl mercury arsenate and phenyl mercury acetate in concentrations of 1:600 to 1:50. A single application of these materials at any of the concentrations used caused no perceptible injury, but with subsequent applications the foliage became yellowish and in some cases the young foliage became curled and crinkled. As a result of this injury these materials were not used in further control tests.

SUMMARY

A bacterial disease of English ivy, *Hedera helix* L., causing a leaf spot and stem canker is described. From the results of inoculations on 12 horticultural varieties, all were found susceptible. The incubation period varied from 5 to 21 days, depending on the temperature, humidity, and age and type of tissues inoculated. Young tissues are more easily infected than old ones, and the incubation period is proportionately shorter.

The causal organism (*Bacterium hederae* Arnaud) has been isolated and its pathogenicity proved by numerous inoculation experiments. Wounds are not necessary for infection, which is evidently stomatal.

The morphological, cultural, and physiological studies of the pathogene are described in detail and a technical description is given. The index number is 5322–3115–1222.

Synergism with 1 associated bacterial organism and anergism with 9 other associated bacterial organisms were noted.

Various methods of control are suggested.

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GROWTH OF CHICKENS AS A FUNCTION OF FEED CONSUMPTION [1]

By Harry W. Titus, *biological chemist*, Morley A. Jull, *senior poultry husbandman*, and Walter A. Hendricks, *junior biologist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The relationship between the weight of feed consumed by a growing animal and the resulting gain in live weight is one of both practical importance and theoretical interest. As a result of a large number of scattered observations, much information has been obtained regarding the factors which influence the economy of gains made by domestic animals, but the information so obtained has never been fully organized. Students of animal nutrition have realized for a long time the need of more exact knowledge of this kind; nevertheless it was not until about 10 years ago, when Spillman (*4*) [2] suggested the use of the equation of the curve of diminishing increment for expressing the relationship between feed consumption and live weight, that, from the point of view of the present writers, the first real advance was made.

In previous papers Jull and Titus (*3*) and Titus (*5*) showed that in crossbred chickens and ducks the equation of the curve of diminishing increment expresses with a high degree of accuracy the relationship between feed consumption and live weight over an appreciable interval of growth. The data presented in these earlier papers, however, did not constitute a rigorous test of the relationship because the same diet was not fed from the first feeding until the conclusion of the experiment, and because a sufficiently long interval of growth was not investigated. Furthermore, as a result of the small number of data involved and the lack of a wholly suitable method of fitting the equation, considerable doubt remained as to the true significance of some of the parameters of the equation.

The experiment described in the present paper was planned for the purpose of (1) obtaining much more extensive and critical data, (2) studying the effect of sex on the utilization of feed, and (3) determining the effect of different levels of feed intake on the relationship between feed consumption and live weight. The plan of the experiment provided for (1) feeding the same diet, but at different graduated levels of intake, to 7 pens of males, as well as to 7 pens of females, for a period of 52 weeks; (2) collecting the pertinent data on feed consumption, live weight, and mortality; and (3) making a mathematical analysis of the data.

EXPERIMENTAL MATERIAL AND METHODS

Approximately 560 chicks were hatched in electric incubators February 13, 1930, at the United States Animal Husbandry Experiment Farm, Beltsville, Md. The eggs were obtained from a flock of

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[2] Reference is made by number (italic) to Literature Cited, p. 835.

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Barred Plymouth Rock females mated to Rhode Island Red males. This mating was employed in order to make use of the sex-linked barring factor which enables one to separate the sexes at hatching time. After removal of the weak and otherwise unsuitable chicks, there remained 265 males and 244 females. These were distributed among 14 pens so that there were 7 pens containing 37 males each and 7 pens containing 34 females each; the remaining chicks were discarded.

The chicks were brooded under electrically heated brooders in a series of pens in a hot-water-heated brooder house. After they were a few days old they were allowed the freedom of small run yards adjoining the pens. By means of this arrangement the chicks had access to direct sunlight whenever the weather permitted. The floors of the pens and run yards were constructed of concrete.

Approximately 10 percent of the chicks developed perosis (*7*, *8*) or other abnormalities and were killed as soon as such abnormalities were observed. During the course of the experiment, as the chicks became larger, others were removed to prevent crowding in the pens.

THE DIET

The following diet, to which 1.5 percent of cod-liver oil was added, was fed in the form of a dry mash to all 14 pens of chicks from the first feeding until the end of the experiment

	Percent
Yellow corn meal	40.0
Ground wheat	22.0
Corn gluten meal	10.0
Dried buttermilk	10.0
Meat scraps (55 percent protein)	10.0
Special steamed bone meal	3.0
Alfalfa-leaf meal	2.5
Yeast preparation [3]	2.0
Common salt	.5
Total	100.0

This diet, as the average of a number of analyses of different lots of it showed, contained approximately 10.4 percent of moisture, 7.1 percent of ash, 4.9 percent of ether-extractable material, 19.4 percent of crude protein, 2.6 percent of crude fiber, and 55.6 percent of nitrogen-free extract.

LEVELS OF FEED INTAKE

One pen of males and 1 pen of females were given all the feed they would eat, and the other 6 pens of each sex were fed at different lower levels of feed intake. Accurate feed-consumption data obtained over an extended period were not available for this particular crossbreed of chickens. Accordingly, in order to determine how much feed to give to each of the 12 pens of chicks that were to be kept on the lower levels of feed intake, use was made of data previously obtained by the writers with several groups of Rhode Island Red chicks in which the sexes had not been separated. From the average ad libitum feed consumption of these chicks, tables were prepared which gave the weight of feed, per chick, for each day in order that the relative levels of feed intake for the 6 pens of each sex would be

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[3] A commercial yeast preparation made by drying a suspension of yeast on corn meal; it contains approximately 50 percent, by weight, of dried yeast.

87.5, 75.0, 62.5, 50.0, 37.5, and 25.0 percent, respectively, of this arbitrarily chosen standard.

The quantity of feed consumed, per chick, by the pen of females which was allowed to eat all the feed that it desired was approximately equal to the ad libitum feed consumption of the Rhode Island Red chicks mentioned previously, but the quantity consumed, per chick, by the corresponding pen of males was appreciably greater. Thus, although the relative levels of feed intake (table 1) were not the same for the corresponding pens of males and females, the absolute levels (actual quantity) of feed intake were practically the same, except for the pen of males and the pen of females which were given all they would consume.

TABLE 1.—*Levels of feed intake of the 7 pens of males and the 7 pens of females*

Males			Females		
Pen no.	Level of feed intake as percentage of the average ad libitum feed consumption of Rhode Island Reds [a]	Relative level of feed intake, as percentage [b] of the average ad libitum feed consumption of crossbred males	Pen no.	Level of feed intake as percentage of the average ad libitum feed consumption of Rhode Island Reds [a]	Relative level of feed intake, as percentage [b] of the average ad libitum feed consumption of crossbred females
	Percent	*Percent*		*Percent*	*Percent*
84	127.5	100.0	85	102.2	100.0
86	87.5	68.6	87	87.5	85.4
88	75.0	58.9	89	75.0	73.5
90	62.5	49.0	91	62.5	60.9
92	50.0	39.2	93	50.0	49.2
94	37.5	29.4	95	37.5	36.8
96	25.0	19.6	97	25.0	24.5

[a] Sexes not separated

[b] Levels of feed intake were computed each week, and the averages for the first 36 weeks were taken

The females in pens 85, 87, 89, and 91 began laying during the twentieth, twenty-third, twenty-fourth, and twenty-seventh weeks, respectively, of the experiment, and for a time an attempt was made to "correct" the feed consumption by subtracting 40 grams of feed for each egg laid (*6*). By the thirty-sixth week the egg production had increased to such an extent in pens 85, 87, and 89 that the writers believed it was no longer possible to "correct" the feed consumption with a sufficient degree of accuracy, and so these three pens were discontinued at the end of the thirty-sixth week.

EXPERIMENTAL DATA

Records were kept of the quantities of feed consumed per chick per week, of the live weights of the chicks at the end of each week, and of the mortality in each pen.

The experimental data on feed consumption and live weights are too voluminous for presentation here. However, the discussion centers around, and most of the conclusions depend on, a relatively small number of constants derived from the data. These constants are given in the discussion which follows.

Since mortality data are of considerable importance in interpreting the results of feeding experiments, table 2 is given. In general the number of birds that died was very small except in the pens receiving less than 50 percent of the ad libitum level of feed.

TABLE 2.—*Number of deaths*[a] *occurring in each pen during each 4-week period*

MALES

Pen no.	Number of deaths during—													Total[b] number of deaths
	First 4 weeks	Second 4 weeks	Third 4 weeks	Fourth 4 weeks	Fifth 4 weeks	Sixth 4 weeks	Seventh 4 weeks	Eighth 4 weeks	Ninth 4 weeks	Tenth 4 weeks	Eleventh 4 weeks	Twelfth 4 weeks	Thirteenth 4 weeks	
84	0	0	0	0	1	0	0	0	0	0	0	0	0	1
86	1	0	0	0	0	0	0	1	0	0	0	0	0	2
88	1	0	0	0	0	0	0	0	0	0	0	0	1	2
90	2	0	0	0	0	0	0	0	0	2	2	0	1	7
92	0	0	0	1	0	0	0	3	3	6	2	2	0	17
94	3	1	0	0	0	1	0	1	6	6	3	0	0	21
96	17	1	0	5	2	2	3	2	2	[c] 1	- -	- -	---	35

FEMALES

Pen no.	First 4 weeks	Second 4 weeks	Third 4 weeks	Fourth 4 weeks	Fifth 4 weeks	Sixth 4 weeks	Seventh 4 weeks	Eighth 4 weeks	Ninth 4 weeks	Tenth 4 weeks	Eleventh 4 weeks	Twelfth 4 weeks	Thirteenth 4 weeks	Total[b] number of deaths
85	0	0	0	2	0	0	0	0	0	([d])	----	- -	- -	2
87	0	0	0	0	0	0	0	1	0	([d])	----	-	-	1
89	1	0	0	0	0	0	0	1	0	([d])	----		- -	2
91	1	0	0	1	0	0	0	0	0	0	0	0	0	2
93	1	0	0	0	0	2	0	0	0	0	1	0	0	4
95	0	0	1	0	0	0	0	0	3	2	1	2	1	10
97	9	0	0	2	0	2	1	1	7	[e] 7	-----		- - -	20

[a] Exclusive of perosis, which does not directly affect growth

[b] There were 37 chicks in each pen of males and 34 chicks in each pen of females at the beginning of the experiment.

[c] This pen was discontinued at the end of the thirty-eighth week because of excessive mortality.

[d] This pen was discontinued at the end of the thirty-sixth week because the egg production had increased to such an extent that as far as the relation between feed consumption and growth was concerned, the data were of questionable value.

[e] This pen was discontinued at the end of the thirty-seventh week because of excessive mortality.

FITTING THE EQUATION OF THE CURVE OF DIMINISHING INCREMENT TO THE EXPERIMENTAL DATA

The equation of the curve of diminishing increment was fitted to the feed-consumption and live-weight data obtained from each of the 14 pens. This equation may be written in two ways, of which one is

$$W = A - BR^F \tag{1}$$

in which, according to Spillman's hypothesis (*4*),

W = the live weight for any corresponding feed consumption, F;
A = the maximum live weight attainable;
B = the difference between A and the initial live weight, i.e., the total gain in live weight possible;
R = the Spillman ratio, which is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed (thus, if one unit of feed produces a gain of 0.30 kilogram and the next a gain of 0.27 kilogram,

$$R = \frac{1}{\frac{0.30}{0.27}} = \frac{0.27}{0.30} = 0.9\text{), and}$$

F = the cumulative feed consumption.

Another form in which this equation may be written is

$$W = A - B\epsilon^{-kF} \tag{2}$$

in which W, A, B, and F have the same significance as before, ϵ is the base of the natural system of logarithms, and k is a constant which is related to R by the following equation:

$$R = \epsilon^{-k} \tag{3}$$

The first step in fitting this equation to the data for each pen was to determine the approximate values of the parameters A, B, and k by the rapid method recently described by Hendricks (*1*). After having obtained the approximate values of A, B, and k, the writers employed the following adjustment equation to determine the corrections to be made to the approximate values of the parameters:

$$\frac{\partial f}{W_o \partial A_o}\alpha + \frac{\partial f}{W_o \partial B_o}\beta + \frac{\partial f}{W_o \partial k_o}\kappa = \frac{W - W_o}{W_o} \tag{4}$$

in which

$$\frac{\partial f}{\partial A_o} = 1, \quad \frac{\partial f}{\partial B_o} = -\epsilon^{-k_oF}; \text{ and } \frac{\partial f}{\partial k_o} = FB_o\epsilon^{-k_oF}$$

and α, β, and κ are corrections to be made to A_o, B_o, and k_o, respectively, which are approximations, previously obtained, of the constants A, B, and k; and W and W_o are, respectively, the corresponding observed and calculated live weights.

The corrected values of A, B, and k were readjusted until the corrections became negligible. In most cases only a single adjustment of the parameters was required because the rapid method gave values of the parameters which required only small corrections. The values of R were then calculated by means of the relationship between k and R, i.e., $R = \epsilon^{-k}$. The unit of feed weight, as well as the unit of live weight, used in this study is the kilogram. In applying the various equations, all weights should be expressed in kilograms.

The writers consider the adjustment equation used in this paper, i.e., equation 4, to be superior to the one previously used by Jull and Titus (*3*), for when equation 4 is used, the sum of the squares of the relative residuals is reduced to a minimum, whereas when the other equation is used, the sum of the squares of the absolute residuals is reduced to a minimum.

THE PARAMETERS AND DERIVED CONSTANTS

In order to summarize as briefly as possible the chief numerical results of fitting the equation of the curve of diminishing increment to the data on feed consumption and live weight, the parameters of this equation, as well as several derived constants, have been tabulated in table 3.

TABLE 3.—*Values of the constants of the live weight-feed consumption equations (curve of diminishing increment) and the coefficients of deviation of the observed from the calculated live weights for each of the 14 pens of chicks*

MALES

Pen no	Relative level of feed intake [a]		Probable error of *A*	*B*	Probable error of *B*	*k*	Probable error of *k*	*R* [b] (or ϵ^{-k})	*kB* [c]	Probable error of *kB*	Coefficient of deviation [d]
	Percent	*Kilograms*	*Kilogram*	*Kilograms*	*Kilogram*						*Percent*
84	100.0	3.677	±0.031	3.642	±0.030	0.0925	±0.0015	0.9116	0.337	±0.003	4.52
86	68.6	2.956	±.010	2.923	±.010	.1203	±.0007	.8867	.351	±.001	1.56
88	58.9	2.621	±.013	2.588	±.013	.1387	±.0012	.8705	.359	±.002	2.45
90	49.0	2.235	±.010	2.202	±.010	.1627	±.0013	.8499	.358	±.002	1.99
92	39.2	1.654	±.012	1.620	±.012	.2041	±.0024	.8154	.331	±.002	3.00
94	29.4	.985	±.009	.951	±.009	.3719	±.0074	.6894	.354	±.005	5.24
96	19.6	.638	±.009	.606	±.009	.5159	±.0141	.5970	.312	±.005	4.62

FEMALES

Pen no	Relative level of feed intake [a]		Probable error of *A*	*B*	Probable error of *B*	*k*	Probable error of *k*	*R* [b] (or ϵ^{-k})	*kB* [c]	Probable error of *kB*	Coefficient of deviation [d]
85	100.0	2.165	±0.023	2.131	±0.022	0.1598	±0.0036	0.8523	0.341	±0.005	4.40
87	85.4	2.362	±.027	2.330	±.027	.1489	±.0031	.8617	.347	±.004	3.72
89	73.5	2.163	±.026	2.132	±.026	.1681	±.0033	.8452	.358	±.003	3.53
91	60.9	1.974	±.009	1.941	±.009	.1800	±.0015	.8353	.349	±.002	2.15
93	49.2	1.641	±.006	1.608	±.006	.2190	±.0014	.8033	.352	±.001	1.64
95	36.8	1.233	±.010	1.201	±.009	.2804	±.0033	.7555	.337	±.002	2.99
97	24.5	.645	±.008	.613	±.008	.4778	±.0095	.6201	.293	±.003	3.22
General mean [e] of *kB* for the 14 pens									.347	±.001	

---

[a] The level of feed intake is expressed as the percentage of the ad libitum feed consumption

[b] The numerical value of ϵ^{-k} is equal to the inverse ratio of the gains in live weight resulting from any 2 successive units of feed consumed. This ratio is referred to as the Spillman ratio. Since $R=\epsilon^{-k}$, the probable error of k is also the probable error of $ln\ 1/R$

[c] The numerical value of kB is equal to the maximum efficiency of the feed in producing gains in live weight. It is the value of the left-hand member of $dW/dF=kB^{-kF}$ when $F=O$

[d] The coefficient of deviation of the observed live weights from the calculated live weights The coefficient of deviation is analogous to the familiar coefficient of variation. In this case it was calculated by means of the formula

$$C \text{ of } D = \sqrt{\frac{\Sigma\left[\frac{100(x-x_c)}{x_c}\right]^2}{N-3}},$$

in which x represents an observed value and x_c represents the corresponding calculated value. To show the analogy between the 2 a comparison may be made of the formula for the coefficient of deviation in the case of a 1-constant equation with that for the familiar coefficient of variation. The formula of the former is

$$C \text{ of } D = \sqrt{\frac{\Sigma\left[\frac{100(x-x_c)}{x_c}\right]^2}{N-1}}$$

and the formula of the latter may be written

$$C \text{ of } V = \sqrt{\frac{\Sigma\left[\frac{100(x-\bar{x})}{\bar{x}}\right]^2}{N-1}} \text{ since } C \text{ of } V = \frac{100\sigma}{\bar{x}} \text{ and } \sigma = \sqrt{\frac{\Sigma(x-\bar{x})}{N-1}}$$

---

[e] Weighted on the basis of the probable errors.

[f] If the values of the maximum efficiency of the feed in the case of pens 96 and 97 are omitted, the general mean maximum efficiency of the feed becomes 0.350±0.001.

INTERPRETATION AND DISCUSSION OF THE EXPERIMENTAL RESULTS

RELATIONSHIP BETWEEN FEED CONSUMPTION AND LIVE WEIGHT AS EXPRESSED BY EQUATION OF CURVE OF DIMINISHING INCREMENT

A comparison of the observed live weights with those calculated by means of the equation of the curve of diminishing increment showed that this equation describes the relationship between feed consumption and live weight with a high degree of accuracy.

To illustrate graphically the excellent agreement between the observed and calculated live weights, the observed average live weights of the cockerels in pens 84 and 86 are plotted, together with the fitted curves, in figures 1 and 2, respectively. Pen 84 was selected as being representative of one of the poorer fits of the equation to the experimental data, whereas pen 86 was selected as being representative of one of the better fits. In the case of pen 84 some of the deviations of the observed live weights from the curve are rather large, but in no instance do they exceed 12 percent of the corresponding calculated values, and the coefficient of deviation is only ±4.52 percent. In the case of pen 86 the largest deviation is less than 7 percent, and the coefficient of deviation has the very low value of ±1.56 percent.

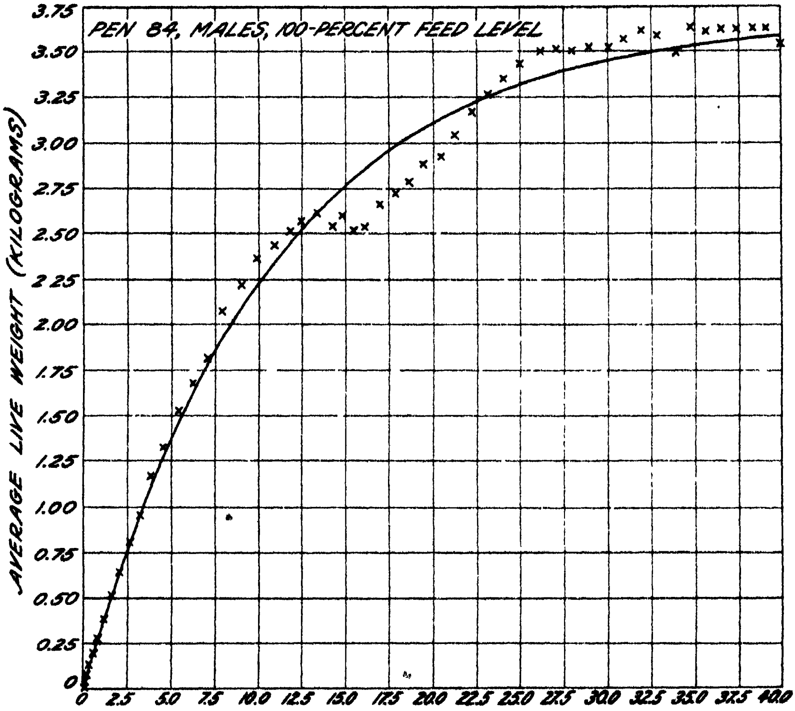

FIGURE 1.—A representative example of one of the poorer fits of the equation of the curve of diminishing increment to the experimental data. The smooth curve was plotted by means of the equation

$$W=3.67695-3.64207e^{-0.0925462F} \text{ (kg)}$$

The crosses represent observed average live weights plotted against cumulative feed consumption.

The coefficients of deviation of the observed from the calculated live weights for all 14 pens are given in table 3. The low values of these coefficients are further evidence of the close agreement between the observed and the calculated live weights, and hence of the ability of the equation of the curve of diminishing increment to express accurately the relationship between live weight and feed consumption.

It was of considerable interest to note that for those pens in which egg production did not complicate the picture most of the deviations, when expressed in percentage, were rather small, except in the case of the 2 pens in which the chicks were allowed to eat all they wanted and the 4 pens on the 2 lowest levels of feed intake. This at least indicates the practicability, as well as the desirability, of controlling, according to a predetermined schedule, the feed intake of animals in comparative feeding experiments, so that the feed consumed will be somewhat less than they would eat of their own free will but at least 50 percent of the quantity that they would normally be expected to eat. A level of feed intake equal to approximately 70 percent of the ad libitum level is recommended because in most cases the animals receiving feed at this level of intake may be expected to eat all that is fed them unless the feed is unpalatable, and because, as is shown later, the value of the Spillman ratio for this level is not so greatly different from its value for the ad libitum level, whereas below the 70-percent level the value of the Spillman ratio decreases with increasing rapidity as the level of feed intake is decreased.

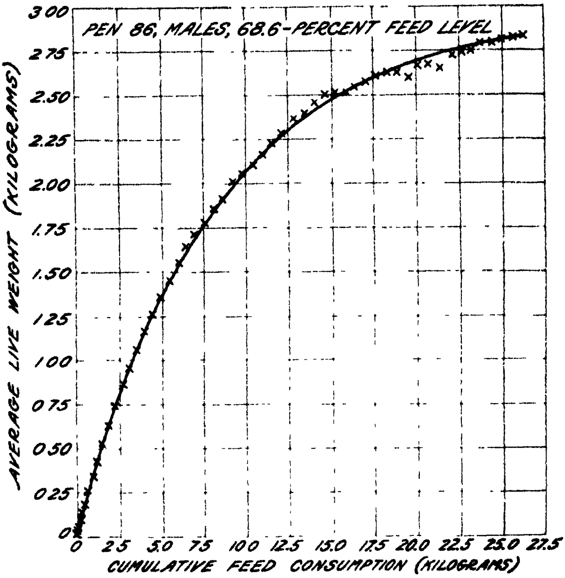

FIGURE 2.—A representative example of one of the better fits of the equation of the curve of diminishing increment to the experimental data. The smooth curve was plotted by means of the equation

$$W=2.95629-2.92281e^{-0.1202525F}\ (\text{kg}).$$

The crosses represent observed average live weights plotted against cumulative feed consumption.

Titus and Hendricks (*9*) have recorded the observation that when chicks are fed at different levels of intake varying from about 40 percent of ad libitum to ad libitum consumption, the live weights of less than approximately 500 grams may be expressed by a single equation relating live weight to feed consumption, regardless of the level of intake. At first the reason for this phenomenon was not clearly evident, but the present study indicates that this follows as a result of one of the properties of the equation of the curve of diminishing increment. Figures 3 and 4 illustrate this point. The curves of the fitted equations are plotted in figure 3 for males and in figure 4 for females. An examination of these curves shows that those for the levels of feed intake above 48 percent of the ad libitum feed consumption almost coincide, in the case of both the males and the females, un-

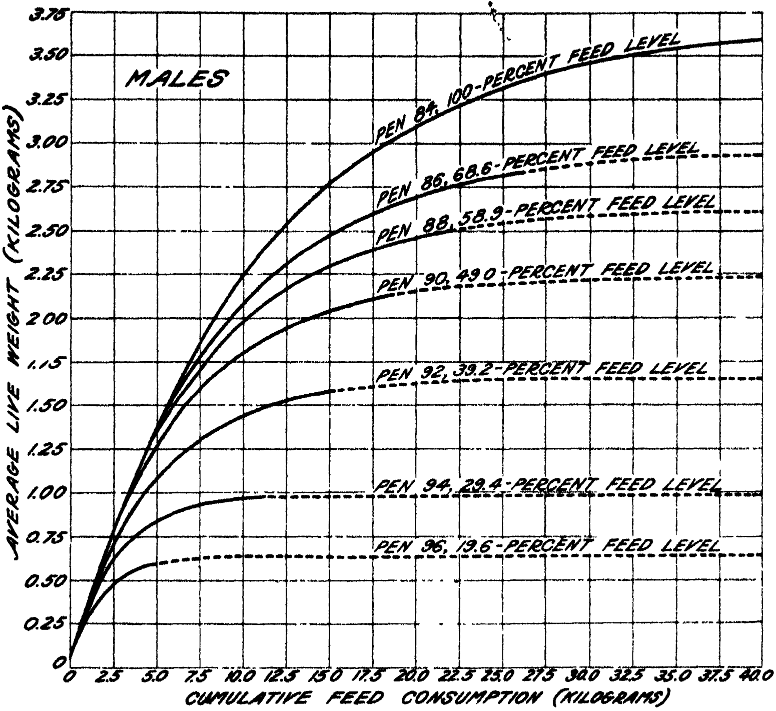

FIGURE 3.—Curves of the fitted equations for the males. Solid lines represent the growth interval studied; broken lines are extrapolations.

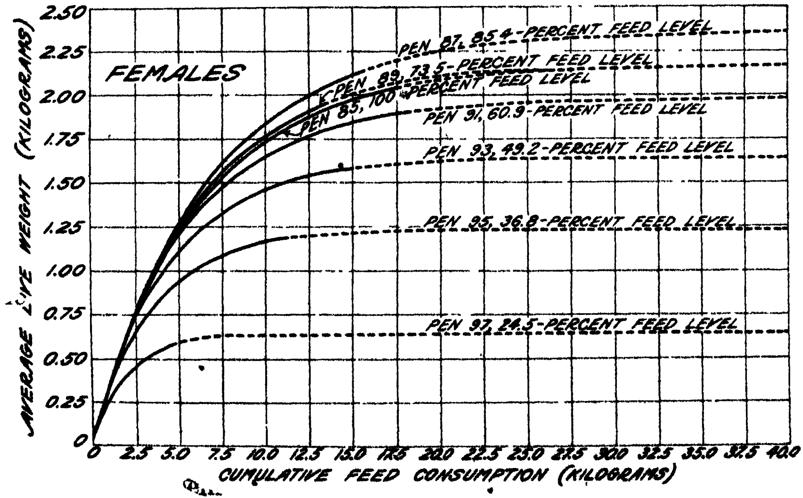

FIGURE 4.—Curves of the fitted equations for the females. Solid lines represent the growth interval studied; broken lines are extrapolations.

til a live weight between 750 and 1,000 grams is reached; and for levels of feed intake above 58 percent, the curves almost coincide until a live weight of nearly 1,250 grams is reached.

Having observed the close dependence of live weight on feed consumption, one may now attempt to determine the cause of the irregularities in the curves showing the relation between feed consumption and live weight (fig. 1) and between age and live weight (fig. 5) for pen 84 and between age and live weight (fig. 6) for pen 85. In figures 7 and 8 the rate of feed consumption of the males and females, respectively, is plotted against age. Inspection of these figures shows that after about the twelfth week the rate of feed consumption by the chicks in pens 84 and 85 was very irregular. And, in general, it is found that there is a direct relation between the irregularities in the live-weight curves and those in the curves depicting the rate of feed consumption. For the sake of comparison, approximations of the curves representing idealized ad libitum rate of feed consumption for males and females are shown (by broken lines) in figures 7 and 8, respectively. When the curves representing the observed rate of feed consumption are compared with the approximations of the idealized curves, the irregularities in the observed rate of feed consumption are brought out in a striking manner.

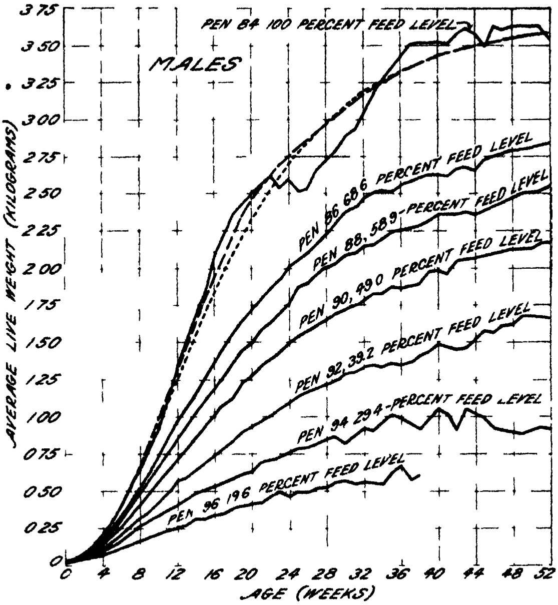

FIGURE 5. Live weights of the males plotted as a function of age. Solid lines represent observed weights, long dash line for pen 84 was obtained by plotting, against age, the average live weights calculated by means of the equation $W = 3.67695 - 3.64207e^{-0.0925462t}$ (kg), short-dash line for pen 84 represents the weights which would have resulted if the feed consumption had followed the curve, shown in figure 7, representing the approximation of the idealized ad libitum rate of feed consumption.

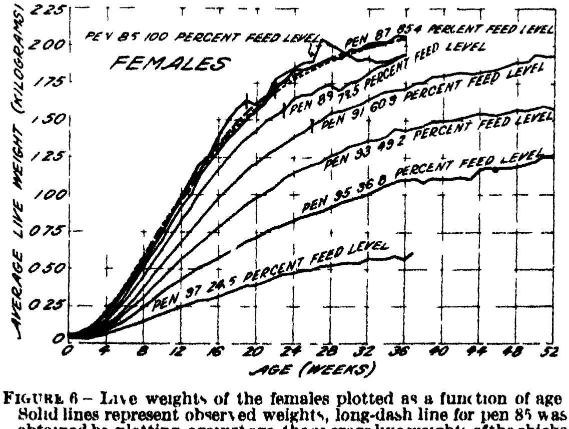

FIGURE 6.—Live weights of the females plotted as a function of age. Solid lines represent observed weights, long-dash line for pen 85 was obtained by plotting, against age, the average live weights of the chicks calculated by means of the equation $W = 2.16495 - 2.13054e^{-0.1598430t}$ (kg), short-dash line represents the weights which would have resulted if the feed consumption of the chicks in pen 85 had followed the approximation of the idealized ad libitum rate of feed consumption curve shown in figure 8. The short lines transecting the curves indicate the approximate age of the females, at beginning of laying, in those pens the feed-consumption data of which were corrected for egg production.

From the explanation just given of the irregularities observed in the curves showing the relation between feed consumption and live weight, and age and live weight, it follows that in conducting a comparative feeding experiment better results are obtained if, in addition to giving all the groups of chickens the same quantity of feed per head, it is arranged that the rate of feed consumption be a fixed percentage of a carefully made approximation of the idealized rate. Obviously, uniformly regular growth can take place only when the rate of feed consumption closely follows a uniformly regular course.

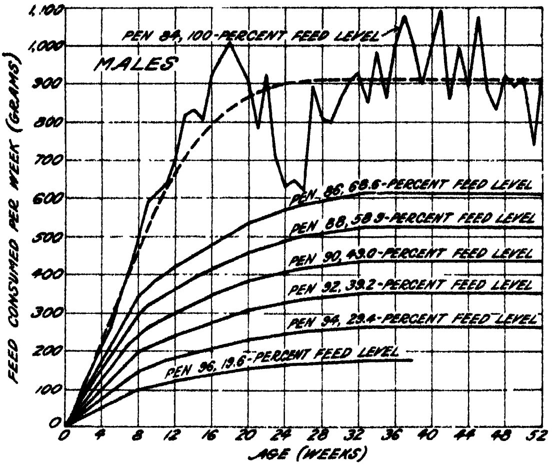

FIGURE 7 --Grams of feed consumed per chick per week in the seven pens of males plotted against age The solid lines represent the observed feed consumption per chick per week, the broken line is an approximation of the curve showing the idealized ad libitum rate of feed consumption of the males in pen 84.

EFFECT OF THE LEVEL OF FEED INTAKE ON THE NUMERICAL VALUE OF THE SPILLMAN RATIO

According to the data presented in table 3, the Spillman ratio (R in equations 1 and 3) decreases in magnitude as the relative level of feed intake is decreased. It now remains to determine the nature of the relationship between the relative level of feed intake and the numerical value of R. If these two variables are plotted against each other, as in figure 9, it at first appears that the relationship is expressible by the equation of the curve of diminishing increment. However, if a calculation is made of the values of R when the unit of feed consumption is 10 kilograms instead of 1 kilogram, and these new values are plotted against the relative levels of feed intake, the sigmoid shape of the curve at once becomes apparent.

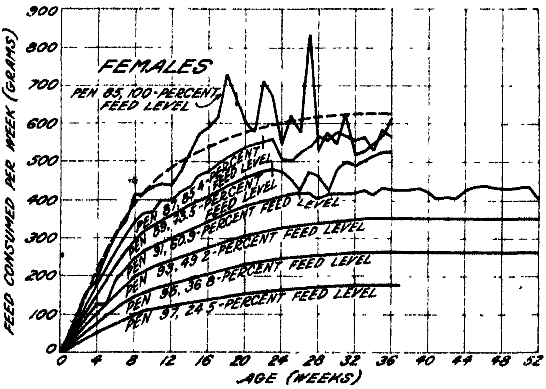

FIGURE 8 —Grams of feed consumed per chick per week in the seven pens of females plotted against age. The solid lines represent the observed feed consumption per chick per week; the broken line is an approximation of the curve showing the idealized ad libitum rate of feed consumption of the females in pen 85. The short lines transecting the curves indicate the approximate age of the females, at beginning of laying, in those pens the feed-consumption data of which were corrected for egg production.

Because of the sigmoid shape of the curve (under the conditions just stated) it was decided to fit the equation

$$ln\frac{y}{a-y}=k(x-b) \tag{5}$$

to the data. When this was done it was found that an excellent fit was obtained, since in no case did the observed values differ from the calculated values by more than 3 percent and the coefficient of deviation was only ±2.13 percent in the case of the males and ± 0.95 percent in the case of the females. The results obtained by fitting equation 5 to the two sets of data are given in table 4.

TABLE 4.—*Effect of the level of feed intake on the numerical value of the Spillman ratio, R*

Males					Females				
Relative level of feed intake [a] (percent)	Spillman ratio, R (or ϵ^{-k})		Difference between observed and calculated		Relative level of feed intake [a] (percent)	Spillman ratio, R (or ϵ^{-k})		Difference between observed and calculated	
	Observed	Calculated [b]	Absolute	Relative		Observed	Calculated [c]	Absolute	Relative
				Percent					*Percent*
100.0	0.9116	0.9096	+0.0020	+0.22	100.0	0.8523	0.8563	−0.0040	−0.47
68.6	.8867	.8928	−.0061	−.68	85.4	.8617	.8543	+.0074	+.87
58.9	.8705	.8763	−.0058	−.66	73.5	.8452	.8499	−.0047	−.55
49.0	.8499	.8458	+.0041	+.49	60.9	.8353	.8380	−.0027	−.32
39.2	.8154	.7937	+.0217	+2.74	49.2	.8033	.8114	−.0081	−1.00
29.4	.6894	.7103	−.0209	−2.94	36.3	.7555	.7476	+.0079	+1.06
19.6	.5970	.5919	+.0051	+.86	24.5	.6201	.6222	−.0021	−.34
Coefficient of deviation				±2.13	Coefficient of deviation				±.95

[a] Expressed as percentage of the ad libitum feed consumption.

[b] Calculated by means of the equation, $ln\frac{y}{0.91212-y}=0.06568(x-10.2403)$, in which y = the Spillman ratio, R, and x = the level of feed intake

[c] Calculated by means of the equation, $ln\frac{y}{0.85728-y}=0.07689(x-11.8420)$, in which y and x have the same significance as in the preceding footnote

Figure 9 clearly shows the relationship between the Spillman ratio and the relative level of feed intake. Although, as previously stated, this ratio decreases as the level of feed intake is decreased, the rate of decrease is relatively slow until a level of about 50 percent is reached, after which it becomes very rapid. This was one of the reasons that earlier in this paper a level of feed intake equal to 70 percent of the ad libitum level was recommended for use in comparative feeding experiments.

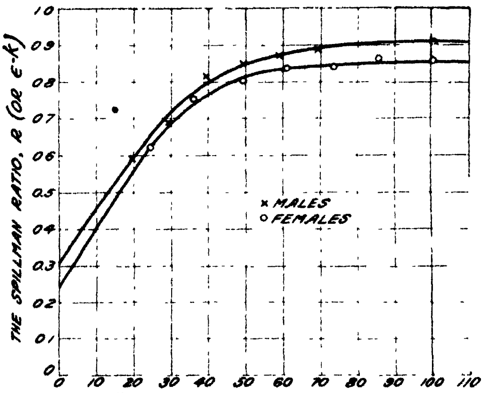

FIGURE 9.—Effect of level of feed intake on the Spillman ratio, R (or ϵ^{-k}). The solid line curves were plotted by means of the equations given in footnotes *b* and *c* of table 4.

By means of the equations given in the footnotes to table 4 one may calculate the value of R with a high degree of accuracy for any relative level of feed intake, at least over the interval studied, i.e., between approximately 20 and 100 percent of the ad libitum feed consumption. In the case of the males it is found that R is equal to 0.9096 for the ad libitum level and 0.8945 for the 70-percent level; and in the case of the females the corresponding values of R are 0.8563 and 0.8476, respectively.

With the exception of swine, the growing animal makes larger gains in live weight when full-fed than when underfed, and makes them more economically. Hence, two very practical questions arise: (1) What constitutes underfeeding? and (2) how serious is a given degree of underfeeding? The answer to the first question is that anything materially less than the ad libitum level of feed intake is underfeeding in the case of the growing animal. The second question can be answered satisfactorily only when the case is accurately specified. Nevertheless it may be shown, by means of the figures given in the preceding paragraph, that the first 4 kilograms of feed consumed by a growing cockerel, if fed at the 70-percent level, are utilized nearly 97 percent[4] as efficiently as they would be if fed at the 100-percent, or ad libitum, level. In the case of the growing pullet the feed is utilized slightly more than 98 percent as well at the 70-percent level as it is at the 100-percent level.

When an animal is grossly underfed, the situation is quite different. For example, a pullet utilizes her first 4 kilograms of feed only about 83 percent as well at the 40-percent level of intake as at the 100-percent level, and the cockerel utilizes his feed only about 79 percent as well.

EFFECT OF THE LEVEL OF FEED INTAKE ON THE TOTAL GAIN POSSIBLE

The data presented in table 5 show that B, the total gain possible on any given level of feed intake, decreases as the level of feed intake is decreased. This is shown graphically in figure 10, which indicates the mathematical form of the relationship between the two. Too few data are available to enable one to determine the precise form of the equation most suitable for expressing B as a function of level of feed intake; however, to enable one to estimate the numerical value of B for any relative level of feed intake within the range studied, the data were graduated by means of the equation

$$y=a-b\epsilon^{-kx} \tag{6}$$

In table 5 the numerical values of B, calculated by means of this equation, are compared with the observed values. Although the

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[4] For computing the relative efficiency of utilization of the feed when fed at two different levels, a function involving only R is to be preferred to one involving both B and R, since R (table 4) was graduated with much greater precision than B (table 5) A suitable function involving only R may be obtained as follows.

Since $W=A-BR^F$, the initial weight, W_1, is given by the equation $W_1=A-B$. It follows, then, that the gain, G, may be expressed as follows: $G=W-W_1=(A-BR^F)-(A-B)=B(1-R^F)$. Hence, the ratio of the gain resulting from the first 4 kilograms of feed at the 70-percent level of intake to the gain resulting from the same quantity of feed at the ad libitum level is

$$\frac{G_{70}}{G_{100}}=\frac{B_{70}\,(1-R^4_{70})}{B_{100}(1-R^4_{100})}.$$

Since the product of k and B is a constant, $k_{70}B_{70}=$ a constant $=k_{100}B_{100}$; hence

$$\frac{B_{70}}{B_{100}}=\frac{k_{100}}{k_{70}}.$$

Also, since $\epsilon^{-k}=R$, $k=-2.3026\log R$. Hence, one may write

$$\frac{G_{70}}{G_{100}}=\frac{B_{70}(1-R^4_{70})}{B_{100}(1-R^4_{100})}=\frac{k_{100}(1-R^4_{70})}{k_{70}(1-R^4_{100})}=\frac{(-2.3026\log R_{100})\,(1-R^4_{70})}{(-2.3026\log R_{70})\,(1-R^4_{100})}=\frac{(-\log R_{100})\,(1-R^4_{70})}{(-\log R_{70})\,(1-R^4_{100})}=$$

$$\frac{(-\log 0.9096)\,(1-0.8945^{4})}{(-\log 0.8945)\,(1-0.9096^{4})}=\frac{(0.04[illegible]1495)\,(0.3598)}{(0.0[illegible]4197)\,(0.3155)}=\frac{0.01480559010}{0.01527641535}=0.9692, \text{ or approximately 97 percent.}$$

agreement between the two is not so good as is to be desired, especially in the case of the males, one can interpolate by means of equation 6 the expected total gain for any level of feed intake with a fair degree of accuracy.

Table 5 shows that the calculated value of *B* for the pen of males and the pen of females which ate all the feed they wanted is appreciably greater than the observed value in both cases. This, no doubt, is due in part to the fact that the chicks in these two pens wasted some of the feed given them. The value of *B* for the pen of males on the 29.4-percent level of feed intake is out of line with the values of this parameter for the other pens of males; likewise the value of *A*, the maximum average live weight attainable, is out of line. The reason for these apparent discrepancies is not known.

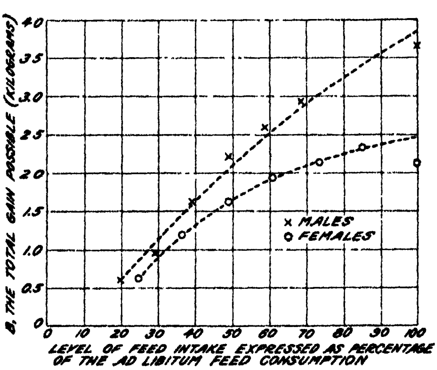

FIGURE 10.—Effect of level of feed intake on *B*, the total gain possible. The dash-line curves were plotted by means of the equations given in footnotes *b* and *c* of table 5.

TABLE 5.—*Effect of level of feed intake on numerical value of B, total gain possible*

Males					Females				
Relative level of feed intake [a] (percent)	*B* (total gain possible)		Difference between observed and calculated		Relative level of feed intake [a] (percent)	*B* (total gain possible)		Difference between observed and calculated	
	Observed	Calculated [b]	Absolute	Relative		Observed	Calculated [c]	Absolute	Relative
	Kilograms	*Kilograms*	*Kilogram*	*Percent*		*Kilograms*	*Kilograms*	*Kilogram*	*Percent*
100 0	3 642	3.845	−0 203	−5 28	100 0	[d] 2.131	2 459	−0 328	[e] −13 34
68.6	2.923	2.837	+ 086	+3.02	85 4	2 330	2 317	+.013	+ 58
58 9	2.588	2 465	+ 123	+5.00	73.5	2 132	2.154	− 022	−1 04
49 0	2.202	2.050	+ 152	+7 42	60 9	1 941	1.919	+ 022	+1.12
39 2	1.620	1 602	+ 018	+1.14	49 2	1 608	1 623	− 015	− 89
29.4	.951	1.113	−.162	−14 54	36.8	1 201	1 195	+ 006	+.54
19.6	.606	.579	+.027	+4.64	24.5	.613	.613		
Coefficient of deviation				±9 37	Coefficient of deviation				[f] ±6 74

[a] Expressed as percentage of ad libitum feed consumption

[b] Calculated by means of the equation, $y=6.97497-7.61302e^{-0.00886543x}$, in which $y=B$, the total gain possible, and $x=$ the level of feed intake.

[c] Calculated by means of the equation, $y=2.77507-4.03429e^{-0.02546420x}$, in which y and x have the same significance as in the preceding footnote.

[d] This value of *B* is obviously out of line with the other values of *B* and, hence, was not used in fitting the equation, $y=a-be^{-kx}$, to the data for the females.

[e] When this value is omitted (for the reason stated in footnote [d]) the coefficient of deviation becomes ±1.12 percent.

[f] As stated in footnote, when the first value in this column is omitted, the coefficient of deviation is reduced from 6.74 to 1.12 percent.

SIGNIFICANCE OF PARAMETERS OF EQUATION OF CURVE OF DIMINISHING INCREMENT WHEN APPLIED TO DATA ON FEED CONSUMPTION AND LIVE WEIGHT

Jull and Titus (*3*), in their earlier study of the growth of chickens in relation to feed consumption, were led to the conclusion that the parameters A and B (M and A, respectively, in their paper) could be considered only as empirical constants. In the present study a much more extensive set of data was obtained, and in the interval between the two investigations a very satisfactory and suitable method of fitting the equation was developed.

A comparison of the observed and calculated live weights showed that the average initial live weights, i.e., the average live weights before any feed was consumed, were reproduced with a high degree of precision by the equation of the curve of diminishing increment. Furthermore, this comparison showed that, on the whole, the observed and calculated average live weights agreed extremely well throughout the growth intervals studied, especially in those pens in which the feed consumption was controlled and in which the level of feed intake was more than 50 percent of the ad libitum level. These considerations lead the writers to the following conclusions regarding the significance of the three parameters of the equation of the curve of diminishing increment.

(1) A represents the maximum average live weight attainable on a given level of feed intake, provided that that level is maintained and that no appreciable fattening occurs. In the case of chickens allowed to eat all they want of an adequate diet, A represents the maximum average live weight attainable, provided no appreciable fattening occurs.

(2) B represents the difference between the maximum average live weight, A, and the average initial live weight; that is, it is numerically equivalent to the average total gain which can be made, if no appreciable fattening occurs.

(3) R, the Spillman ratio, is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed.

Since the first derivative of the equation of the curve of diminishing increment may be written

$$dW/dF = kB\epsilon^{-kF} \qquad (6)$$

or

$$dW/dF = kBR^{F} \qquad (6')$$

it follows that kB is equal to the maximum efficiency of the feed in producing gains in live weight, if this efficiency is defined as the ratio of the gain in live weight to the quantity of feed required to produce the gain. The efficiency of successive units of feed decreases in geometrical progression and the magnitude of the decrease is determined by the Spillman ratio, R (or ϵ^{-k}).

In regard to its significance, kB should not be confused with kA, which the writers proposed in an earlier paper (*2*) as a measure of feed efficiency. The former represents the maximum efficiency which a feed may actually have and the latter represents the maximum efficiency which it would have if the animal to which it is fed had an inital live weight of zero. The value of kA is dependent on the maximum live weight attainable by the animal, whereas the value of kB is dependent on both the maximum and initial live weights, since

$$B = A - w_1 \text{ (}w_1\text{ being the initial live weight).}$$

The values of the maximum efficiency of the feed (kB) are given in table 3 for each of the 14 pens. Although there appears to be a slight tendency for the maximum efficiency to increase at first and then to decrease as the level of feed intake is decreased, there is a marked agreement among the values. If the values for the two pens on the lowest absolute level of feed intake are eliminated, the weighted mean value of kB is 0.350 ± 0.001 and the range is from 0.331 to 0.359.

The various numerical values of A, B, and R in the equation of the curve of diminishing increment and the parameters of the equations which are given for expressing the relationship between (1) R (the Spillman ratio) and the level of feed intake and (2) B (the total gain possible) and the level of feed intake hold only for the particular diet fed and for the particular crossbreed of chicken used. However, it is reasonably certain that, with suitable values for the parameters, the several equations used in this study will hold for any adequate diet and for any breed of chickens.

EFFECT OF SEX ON UTILIZATION OF FEED

If, for corresponding absolute levels of feed intake, one compares the numerical values of the Spillman ratio for the two sexes, he finds that for the first three of the levels less than the ad libitum level the values of R are greater for the males than they are for the females, but on the two lowest absolute levels the opposite is true. If it is assumed that the product kB is constant regardless of sex or level of feed intake (and the values of this product given in table 3 seem to indicate that this may be the case), one is led to the conclusion that on the higher absolute levels of feed intake the males of this crossbreed are more efficient in the utilization of feed than the females, but on the lower levels the latter are the more efficient.

Even if one does not make this assumption regarding the constancy of the product kB, the conclusion still appears to hold for the following reasons:

(1) The efficiency of the feed is given by the equation

$$\text{Efficiency} = dW/dF = kBR^F;$$

(2) The Spillman ratio, R, decreases at a greater rate than does the product kB after the latter's apparent maximum is reached;

(3) The values of the Spillman ratio on the higher levels of feed intake are greater for the males than for the females; and

(4) On the two lowest levels the opposite of the preceding statement is true.

If, instead of comparing the efficiency of the males and females on the basis of the absolute levels of feed intake, one makes the comparison on the basis of the relative levels of feed intake, it is found that the males are the more efficient for all the relative levels studied.

SUMMARY AND CONCLUSIONS

Extensive and critical data were obtained on the relationship between feed consumption and live weight in the case of crossbred chickens (Rhode Island Red males mated to Barred Plymouth Rock females). Seven pens of males and seven pens of females were fed at different levels of feed intake, including the ad libitum and six lower levels, for a period of 52 weeks. The weight of feed consumed per chicken per week and the average live weight of the chickens at the end of each week were obtained.

By means of a suitable method, the equation of the curve of diminishing increment was fitted to the data recorded for each of the 14 pens of chickens. It was found that the average live weights computed by means of this equation agreed very closely with the observed average live weights. It was also found that a rational meaning could be assigned to the three parameters of this equation.

It was possible to describe accurately, by means of a simple equation, the relationship between the level of feed intake and the Spillman ratio. It is recommended that in comparative feeding experiments all the groups be fed at a level of feed intake equal to 70 percent of an approximation of the idealized ad libitum feed consumption. At this level the value of the Spillman ratio is nearly as large as it is at the ad libitum level. Under these conditions the several groups would eat the same quantity of feed, unless it were unpalatable, and the feed consumption would follow a definite course. The latter point is of importance since uniformly regular growth can take place only when the feed consumption follows a uniformly regular course.

The following conclusions are drawn:

The equation of the curve of diminishing increment, $W=A-BR^F$, describes with a high degree of accuracy the relationship between feed consumption and live weight over a wide range of levels of feed intake.

A in the above equation represents the maximum live weight attainable on a given level of feed intake, provided this level is maintained and no appreciable fattening occurs.

B in the above equation represents the maximum gain possible under the conditions just stated.

R, the Spillman ratio, is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed.

The relationship between level of feed intake and the numerical value of R is expressible, over the range of levels studied, by the equation,

$$ln \frac{y}{a-y}=k(x-b), \text{ in which}$$

y = the Spillman ratio, x = the relative level of feed intake, and a, k, and b are constants.

The relationship between level of feed intake and the numerical value of B is expressible, for the range of levels studied, with a fair degree of accuracy by the equation

$$y=a-b\epsilon^{-kx}, \text{ in which}$$

$y=B$, the total gain possible; x = the relative level of feed intake, and a, b, and k are constants.

On the higher absolute levels of feed intake the males of the crossbreed studied are more efficient than the females in their utilization of feed for growth, whereas on the very low absolute levels the opposite is true.

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COMPARISON OF THE PULLORIN AND THE RAPID WHOLE-BLOOD AGGLUTINATION TESTS FOR PULLORUM DISEASE[1]

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INTRODUCTION

The control of pullorum disease is based on the diagnosis of the carrier fowl and its elimination from breeding. Various tests are at present in use in different parts of the country for diagnosing the pullorum-disease carrier. The "long" or "tube" agglutination test has been in use since 1913 and has been developed to a considerable degree of refinement. The principal objections to it are its slowness and the comparatively great expense and labor involved in its application. To meet these objections more simple methods have since been devised. Some of these retain the principle of serum-antigen agglutination, but one is based upon the allergic reaction of living tissue. This last is known as "the pullorin test" or "the intradermic test" for pullorum disease. The name "pullorin" refers to the reagent employed.

However, in the 17 years in which it has been known, the pullorin test has not been widely accepted by poultry-disease diagnosticians, many of whom have been skeptical as to its accuracy. The experiments recorded in this paper were conducted with a view to ascertaining more fully the relative diagnostic value of the pullorin test as compared with the rapid whole-blood agglutination test[3] for pullorum disease. The latter test involves the use of a stained antigen. The rapid whole-blood agglutination test was selected for the comparisons because it had the advantages claimed for the pullorin test with regard to simplicity and economy. The experiments also included some corroborative tests by the tube agglutination method.

HISTORICAL REVIEW

Ward and Gallagher[4] in 1917 first reported the development of an intradermic test for pullorum disease. These workers prepared an allergic reagent by inoculating broth with cultures of *Salmonella pullorum*, incubating at 37° C. for 1 month, holding the broth in an ice box for about 6 months, then passing it through a Berkefeld filter, and finally preserving it with 0.5-percent phenol. This product was

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[2] The writer expresses his indebtedness to J. M. Rosell, professeur de bacteriologie a l'Ecole de Medicine Veterinaire de la Province de Quebec et a l'Institut Agricole d'Oka, for preparing pullorin R and administering and interpreting this test in the flock in which it was used; to George W. Stiles, in charge of the U.S. Department of Agriculture branch pathological laboratory at Denver, Colo., for preparing pullorin S, and to W. J. Hall, assistant veterinarian in charge of the Department's branch pathological laboratory, Beltsville, Md., for his valuable assistance in conducting the agglutination test on the flock in which pullorins R and S were used.

[3] SCHAFFER, J. M., MACDONALD, A. D., HALL, W. J., and BUNYEA, H. A STAINED ANTIGEN FOR THE RAPID WHOLE BLOOD TEST FOR PULLORUM DISEASE. Jour Amer. Vet. Med. Assoc. (n s. 32) 79: 236-240 1931.

[4] WARD, A. R., and GALLAGHER, B. A. AN INTRADERMAL TEST FOR BACTERIUM PULLORUM INFECTION IN FOWLS. U.S. Dept. Agr. Bull. 517, 15 pp. 1917.

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injected intradermically into one of the wattles of the fowl to be tested. After 24 hours the injected wattles of infected fowls were swollen, and those of uninfected fowls were not swollen. The swelling of the wattles of reactors increased noticeably in the next 24 hours.

These authors later modified their product by growing the organism in broth for 1 month at 37.5° C., and killing it by heating at 60° for 1 hour in a water bath. They then carbolized it to 0.5 percent. This product was employed without filtration.

The formula thus developed has been largely the basis of most of the commercial and experimental pullorins since used. Pullorins of the type of that first described are usually referred to as noncellular, or cell-free pullorins, whereas those of the second type are known as cellular pullorins.

A number of contemporary workers have investigated the possibilities of the diagnosis of pullorum disease by means of an allergic test. Rettger and Plastridge[5] state: "The beliefs expressed at the 1930 Poultry Congress in London were, with one exception, distinctly unfavorable to the pullorin test." A translation of Rosell's[6] comments on the comparative results between the pullorin test and the stained-antigen, rapid whole-blood test is as follows:

> In a comparative test of the efficiency of this method with those employing different kinds of pullorin, one of which was of the soluble type which I prepared by a new method, the results achieved in collaboration with Drs. Hall and Bunyea were favorable to the whole-blood method which possessed the advantage of requiring only one visit to the poultry plant. This method assuredly provides the more practical means of combating pullorum disease.

EXPERIMENTAL PROCEDURE

The experiments comprised field trials of the pullorin test in comparison with the stained-antigen, rapid whole-blood agglutination test[7] applied to previously untested poultry flocks in the vicinity of Washington, D.C. The plan of this investigation included the use of several commercial brands of pullorin and one or more pullorins prepared by research workers. Arrangements were accordingly made with four nearby flock owners for the application of the comparative tests, which were to be conducted simultaneously. However, neither test was to be set up as the standard by which to judge the merits or defects of the other. The value of a diagnostic test consists in its ability to detect the presence of the elements or processes of disease. Therefore, the criterion proposed in these experiments was that of the laboratory demonstration of *Salmonella pullorum* in the carcasses of reacting fowls selected from the four flocks tested.

In the case of fowls slaughtered from the last three flocks, a tube test was included at autopsy, as having some corroborative value with reference to the rapid whole-blood agglutination test findings.

Five different pullorins were used, two of which were noncellular and three cellular. The two experimental pullorins, R and S, were prepared, respectively, by J. M. Rosell[8] and George W. Stiles. Rosell gives the following information[9] concerning his method of preparation of the cell-free pullorin:

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[5] RETTGER, L. F., and PLASTRIDGE, W. H. PULLORUM DISEASE OF DOMESTIC FOWL. A MONOGRAPH. Conn. (Storrs) Agr. Expt. Sta. Bull. 178, pp. [109]-192 illus. 1932.

[6] ROSELL, J. M. PROGRÈS ET NORMES SUR QUELQUES POINTS D'ACTUALITÉ EN PATHOLOGIE ANIMALE. Rev. Inst. Agr. Oka 7 (3): 100-104. 1933.

[7] SCHAFFER, J. M., MACDONALD, A. D., HALL, W. J., and BUNYEA, H. See footnote 3.

[8] ROSELL, J. M. See footnote 6.

[9] ROSELL, J. M. Informal communication.

Five strains of *Salmonella pullorum* (B.A.I. strains 10, 11, 14, 17, and 20) were used in preparing the pullorin. Each strain was grown separately in flasks containing 20 cc of glucose-peptone broth adjusted to a reaction of pH 7.2. The flasks were incubated for 10 days at 37° C. At the end of this period sufficient sterile sodium hydroxide was added so that the broth gave a clear alkaline reaction, by using phenolphthalein as an indicator. It was again incubated for 3 days at 37° or 40° C. in order to obtain a better autolysis of the cells.

After the 3-day incubation period, the culture were frozen for many hours by the use of dry ice, and then melted at 45° C. This procedure of freezing and melting was repeated twice in order that a more complete autolysis be obtained. Microscopically, smears revealed that most of the cells were autolysed.

After purity tests were made the different culture autolysates were mixed and centrifuged in large centrifugal tubes. The supernatant fluid was removed by decantation. To the reunited sediments 20 cc of 0.75-percent sterile potassium hydroxide was added, and this was then shaken and heated at 50° C. until a gelatinous fluid was obtained. This fluid was then centrifuged and the supernatant fluid was added to the supernatant fluid obtained from the first centrifugation. This liquid was adjusted to a pH of 7.2 by adding N/10 hydrochloric acid.

To obtain a more concentrated pullorin the liquid was submitted to a process of evaporation without heating it over 48° C. The liquid was placed in a shallow porcelain pan which was maintained in a water bath of 48° C. for 5 hours. During this time air was allowed to pass through the liquid by means of small glass pipes, and the air currents from an electric fan passed over the surface of the liquid. By this means 1,000 cc of the fluid was evaporated to 666 cc.

Sufficient carbolic acid was then added to the product so that it would contain 0.3 percent of this preservative. It was then filtered through a Mandler candle and transferred aseptically into 20-cc sterile rubber-stoppered vials.

The pullorin supplied by Dr. Stiles was prepared by him essentially after the modified Ward and Gallagher formula, as follows: Five or six flasks of broth were inoculated with separate strains of *Salmonella pullorum*, incubated for at least 1 month, heated at 60° C. for 2 hours, carbolized, and tested for sterility. They were mixed before use.

No information is available as to the methods used in the manufacture of the several commercial pullorins.

The method employed in determining the presence of *Salmonella pullorum* infection in the slaughtered birds was the same as that used by Bunyea and Hall,[10] namely, the aseptic removal and crushing of the entire ovary or testicle, which was then placed in a culture flask of beef infusion broth containing brilliant-green dye in the proportion of 1 to 50,000. Individual colonies of *S. pullorum* were recovered from subcultures made from the brilliant-green broth cultures onto plain agar. The organisms were identified in each instance in the manner described by Bunyea and Hall.

RESULTS OBTAINED

Table 1 shows the percentage of agreements and disagreements between the reactions (1) to the various pullorins used intradermically in the pullorin test and (2) to results obtained with the rapid whole-blood agglutination test.

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[10] BUNYEA, H., and HALL, W. J. THE RELATION OF AGGLUTINATION REACTION TO SALMONELLA PULLORUM INFECTION IN HENS, AND OBSERVATIONS ON THE DIAGNOSTIC EFFICIENCY OF TEST METHODS. Jour Amer. Vet. Med. Assoc. (n s. 33) 80: 491-496, illus. 1932

TABLE 1.—*Percentages of agreements and disagreements between results of the pullorin test and the rapid whole-blood agglutination test*

Pullorin used		Birds tested	Agreements [1]		Disagreements [1]	
Source and designation	Type		Pullorin +, agglutination +	Pullorin −, agglutination −	Pullorin +, agglutination −	Pullorin −, agglutination +
		Number	*Percent*	*Percent*	*Percent*	*Percent*
Experimental:						
R	Noncellular	218	18.4	49.5	5.5	26.6
S	Cellular	107	23.4	44.9	12.0	19.7
Commercial.						
1	do	224	1.3	66.5	3.6	28.6
2	Noncellular	182	17.0	52.2	14.8	16.0
3	Cellular	129	2.3	71.3	6.2	20.2
Total or average		860	11.8	57.2	7.9	23.0

+ indicates a positive reaction; −, a negative reaction.

From table 1 it may be observed that the total average agreement in both positive and negative cases is 69 percent, whereas the total average disagreements aggregate 31 percent. Marked fluctuations for the various pullorins are plainly evident.

Tables 2 to 4 show comparisons of the results obtained from the pullorin tests, and the rapid whole-blood agglutination tests, based on their agreement or disagreement with bacteriological findings. Tables 3 and 4 include results obtained from the tube agglutination test, used post mortem.

TABLE 2.—*Comparison of results obtained from pullorins R and S and the rapid whole-blood agglutination test, based on their agreement or disagreement with bacteriological findings*

Fowl no.	Reaction of bird to—			Post-mortem findings		Agreement (+) or disagreement (−) between bacteriological findings and—	
	Pullorin R	Pullorin S	Rapid whole-blood agglutination test	Typical lesions noted	*Salmonella pullorum* isolated	Pullorin test	Rapid whole-blood agglutination test
30	Negative		Positive	No	Yes	−	+
414	do		Negative	No	No	+	+
13	Positive		do	Yes	Yes	+	−
70	do		do	No	No	−	+
287	do		do	No	No	−	+
172	do		do	No	No	−	+
422	do		do	No	No	-	+
74	Negative		do	Yes	Yes	−	+
10	do		do	Yes	Yes	−	+
90	do		do	Yes	Yes	−	+
148		Positive	do	Suspicious	No	−	+
101		do	do	do	No	−	+
191		do	do	No	No	−	+
135		do	do	Suspicious	No	−	+
230		Negative	Positive	Yes	Yes	−	+

TABLE 3.—*Comparison of results obtained from commercial pullorins no. 1, no. 2, and no. 3, and the rapid whole-blood and tube agglutination tests, based on their agreement or disagreement with bacteriological findings*

PULLORIN NO. 1

Fowl no.	Reaction of bird to—		Post-mortem findings					Agreement (+) or disagreement (−) between bacteriological findings and—	
	Pullorin test	Rapid whole-blood agglutination test	Tube agglutination test with blood-serum dilutions of—			Typical lesions noted	*Salmonella pullorum* isolated	Pullorin test	Rapid whole-blood agglutination test
			1:25	1:50	1:100				
95	Negative	Positive	Positive	Positive	Positive	No	Yes	−	+
76	do	do	do	do	do	No	No	+	−
83	do	do	do	do	do	No	No	+	−
451	do	do	do	do	do	Yes	Yes	−	+
107	Positive	Negative	Partial	Partial	Partial	No	No	−	+
57	Negative	Positive	Positive	Positive	Positive	No	No	+	−
25	do	do	Slight	Partial	do	No	Yes	−	+
140	do	do	Positive	Positive	Partial	No	No	+	−
82	do	do	do	do	Positive	No	Yes	−	+
PULLORIN NO. 2									
2760	Positive	Positive	Positive	Positive	Partial	No	No	−	−
2766	Negative	do	do	Negative	Negative	No	No	+	−
6308	Positive	do	do	do	do	No	Yes	+	+
9182	do	do	do	Positive	Positive	Yes	Yes	+	+
6332	Negative	do	do	do	Negative	No	Yes	−	+
2756	Positive	do	do	do	Positive	No	No	−	−
2747	Negative	do	do	do	do	No	Yes	−	+
2730	Positive	do	do	do	Negative	Yes	Yes	+	+
6338	Negative	do	do	do	Positive	Yes	Yes	−	+
6376	Positive	do	do	do	do	Yes	Yes	+	+
2759	Negative	do	do	do	Slight	No	No	+	−
6386	Positive	do	do	do	Positive	Yes	Yes	+	+
PULLORIN NO. 3									
170	Negative	Positive	Positive	Positive	Partial	Yes	Yes	−	+
123	do	do	do	do	Positive	No	No	+	−
164	do	do	do	do	do	Yes	Yes	−	+
177	do	do	do	do	do	Yes	Yes	−	+
175	do	do	do	do	Partial	Yes	Yes	−	+
966	do	do	do	do	do	Suspicious	Yes	−	+
993	do	do	do	Partial	Positive	do	Yes	−	+
181	do	do	do	Positive	do	Yes	Yes	−	+
967	do	do	do	do	do	Yes	Yes	−	+
159	do	do	do	do	do	Yes	Yes	−	+
982	do	do	do	do	do	No	Yes	−	+
149	do	do	do	do	Partial	No	Yes	−	+
169[a]	Positive	Negative				No	No	−	+
124	do	do	Negative	Negative	Negative	No	No	−	+
171	do	do	do	do	do	No	No	−	+
156	do	do	do	do	do	No	No	−	+

[a] This bird was found dead; consequently no tube agglutination test was made.

TABLE 4.—*Summary of agreements between the diagnostic test reactions, bacteriological findings, and results of the tube agglutination test used post mortem*

Pullorin used	Fowls examined post mortem	Agreement between pullorin test and—		Agreement between rapid whole-blood agglutination test and—	
		Bacteriological findings	Tube agglutination test	Bacteriological findings	Tube agglutination test
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Experimental					
R	10	20.0		90.0	-
S	5	0.0		100.0	
Commercial					
1	9	45.5	0.0	55.5	100.0
2	12	58.3	58.3	66.7	83.3
3	16	6.3	0	93.8	100.0
Total or average	52	26.0	19.4	81.2	94.4

DISCUSSION

The significant results in tables 2 and 3 and summarized in table 4 are the percentages of agreement between the various tests and the bacteriological findings.

In tables 2 and 3, particular interest centers on the results with commercial pullorum no. 1. The birds used were chiefly pullets. Eight of the nine cases examined post mortem showed no macroscopic lesions of pullorum disease, and *Salmonella pullorum* was isolated from only 4 of the 9 birds. With young fowls it is more difficult to harmonize the serological and bacteriological results than with more mature ones. The corroborative evidence of the tube test in this group gives support to the inference that pullorum infection, though undiscovered in some birds, probably lurked in their bodies.

As already noted, macroscopic lesions of pullorum disease in reactors are sometimes lacking, particularly in young fowls, even when the infection may be demonstrable by bacteriological procedure. Therefore a diagnosis eliminating pullorum disease on the basis of no lesions cannot be made with assurance.

In table 4 there is seen to be excellent average agreement, 94.4 percent, between the rapid whole-blood agglutination test and the tube test, thus supporting the reliability of the rapid test. There is also good agreement, 81.2 percent, between the rapid whole-blood agglutination test and the bacteriological findings. The results obtained by the pullorin test show a much lower percentage of agreement with the bacteriological findings and the tube agglutination test. The evidence thus obtained indicates that the rapid whole-blood test is a more reliable diagnostic agent for pullorum disease than the pullorin test.

SUMMARY AND CONCLUSIONS

Each of four commercial poultry flocks was tested for pullorum disease by the stained-antigen, rapid whole-blood agglutination test and the pullorin test. In addition, the tube agglutination test was used post mortem on the serum of birds from three of the flocks, as a check on the reliability of the rapid whole-blood method. Specimen birds were obtained from these flocks, examined post mortem, studied bacteriologically, and the findings compared with the test findings. In every group the agreement between the rapid whole-blood agglutination test and the bacteriological findings was more favorable than that between the pullorin test and the bacteriological findings. The results of the tube agglutination method, which is of recognized dependability, supported the reliability of the rapid whole-blood agglutination test.

Although the experiments here recorded are of limited scope, the evidence obtained is in accord with the views expressed by other workers, that the pullorin test is not so satisfactory a means of diagnosing pullorum disease as the rapid whole-blood agglutination test.

THE DISTRIBUTION AND CONDITION OF NITROGEN IN THREE HORIZONS OF A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN METAL CYLINDERS[1]

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INTRODUCTION

In a recent paper (*15*) [2] the writer reported the utilization and recovery of nitrogen, phosphorus, and potassium by apple trees grown in metal cylinders for a period of 6½ years. These trees received (each spring) during the last 3 years of growth different combinations of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate. It was shown that the ratio in which nitrogen (as N), phosphorus (as P_2O_5), and potassium (as K_2O) were absorbed from the added salts ($NaNO_3$, $CaH_4(PO_4)_2$, and K_2SO_4) by those trees optimum with respect to growth and reproduction, that is, the trees receiving the NPK and NP treatments, was 3:0.3:1.5 as compared with a 3:8:4 ratio actually applied. This latter ratio was based on the current orchard practice. The great divergence between these ratios indicated the need for information as to the condition of the added nitrogen, phosphorus, and potassium in the soil in order to determine the extent to which these "theoretical" quantities and ratios should be modified as a result of the changes produced by the interaction of the added salts with the soil. The present investigation reports the status of the nitrogen in the three soil horizons in the cylinders from which the trees referred to above were removed.

METHODS OF EXPERIMENTATION

Inasmuch as the detailed plan of this experiment has been reported elsewhere (*3*, *14*, *16*) a brief outline only is necessary.

The soil, the analysis of which has been recorded (*11*), was formed in place by the weathering of limestone to the lower Silurian formations, and is of Trenton origin. The excavation was made on a strip of land 110 by 11 feet adjacent to the college experimental orchard. The history of this land indicates that except for the droppings of cattle no dressings of fertilizer had ever been applied. It may, therefore, be described as a virgin forest soil. The mechanical analysis (*11*) suggests that the surface soil consists of a heavy silt loam underlain by a clay loam which becomes heavier in texture as the depth increases.

The soil was excavated from this strip by a scoop shovel. The layers from each of three horizons, viz, surface (0 to 7 inches), subsurface (7 to 21 inches), and subsoil (21 to 53 inches), were kept separate and each was thoroughly mixed and weighed. The total

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[1] Received for publication Nov. 4, 1933; issued July 1934. Technical Paper no. 613 of the Pennsylvania Agricultural Experiment Station. Presented before the American Society for Horticultural Science at the Boston (1933-34) meeting of the American Association for the Advancement of Science.

[2] Reference is made by number (italic) to Literature Cited, p. 856

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weight of the horizons was 54,180, 109,200, and 249,000 pounds, respectively. Inasmuch as there were 42 cylinders, an equal distribution (by weight) of each layer among the cylinders would require the following quantities of soil to be added to each of the cylinders: Subsoil, 5,930 pounds; subsurface soil, 2,600 pounds; and surface soil, 1,290 pounds. This equal distribution was effected by ascertaining the weight of each of the respective horizons required to fill a steel wheelbarrow similar to those used in highway construction work. Such wheelbarrows were used in filling the cylinders. Following the addition of each wheelbarrow load, uniformity in density of the soil was secured by means of heavy wooden mallets fitted with 3-inch cast-iron pipe handles 5 feet in length. The process of filling the cylinders was completed in the spring of 1920. Uniformity with respect to the nitrogen, phosphorus, and potassium content of the soil in the cylinders at this stage was established by analysis. The mean of 30 determinations for total nitrogen in the original soil is given in the first line of table 1. Any departure from these values greater than the error of analysis (±0.0001 percent) must be attributed to causes resulting from differences in treatment.

The trees were planted in the spring of 1922. Up to the spring of 1924 the system of culture was similar in all cylinders. This consisted of green manuring with buckwheat and rye principally. In May 1924 half the cylinders were seeded with a mixture of Kentucky bluegrass, *Poa pratensis* L., and timothy, *Phleum pratense* L. These cylinders are designated "cylinders under sod." In the remaining half of the cylinders a tillage system was adopted. These latter cylinders are designated "cylinders under cultivation." A distinction must be noted with respect to the additions of nitrogen from 1925 until the end of the experiment in 1927. During these last 3 years of the experiment the cylinders under cultivation received 15.9 grams more nitrogen than the cylinders under sod. The reason for this is that it was then considered necessary to add equal amounts of organic matter to all the cylinders under cultivation. This was accomplished by growing rye outside the cylinders. For further details the paper by Anthony and Clarke (*3, p. 251*) should be consulted. All trees were allowed to grow without the addition of any mineral fertilizer until the spring of 1925, at which time differential treatment with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate was commenced. It is important to note that the conditions of this experiment preclude any erosion by water and practically none by wind.

The sodium nitrate was added according to the following schedule:

	Grams
April 18, 1925	906
May 3, 1926	45
June 7, 1926	453
June 20, 1926	408
May 5, 1927	337
May 18, 1927	338
June 10, 1927	337
Total	2, 824

This total of 2,824 g of sodium nitrate is equivalent to 465.5 grams of elemental nitrogen.

During the period from September 20 to 28, 1927, the trees were dup up and soil samples representative of the three horizons were taken, by the method of successive quartering, from each of the cylinders from which the trees had been removed. These samples were dried at 75° C. and then sieved through a 1-millimeter sieve (*4*) and stored in glass jars in the dark. Analyses of the trees have already been reported (*14*).

Total nitrogen was determined by the Kjeldahl-Gunning method to include the nitrogen of nitrates, a 15-g charge being used for the surface soil and 30-g for the subsurface and subsoil (*4*). Nitric nitrogen was determined by the Devarda alloy method, a 200-g charge being used (*1*). The analytical data in the tables are the means of closely agreeing triplicate determinations. The quantities of ammonia nitrogen and nitrous nitrogen in all horizons were insignificant. All calculations are made on a moisture-free basis.

EXPERIMENTAL DATA

Table 1 gives the percentage and absolute amounts of total nitrogen in each of the three soil horizons; that is, the 0 to 7 inch, the 7 to 21 inch, and the 21 to 53 inch.

TABLE 1.—*Percentages and absolute amounts of total nitrogen in the respective horizons before the trees were planted and at the end of the experiment*

Treatment	Total nitrogen			Absolute amount total nitrogen			
	Surface (0 7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Total (0–53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.08650	0 05003	0 03523	506.1	589.7	946 8	2,042 6
Sod:							
Check	.08219	04600	.03400	477 9	542 5	914 5	1,934.9
PK	.08200	.04700	03498	479 8	554 3	941 4	1,975 5
NPK	.08200	.05160	.03900	478.6	613 2	1,049 0	2,140 8
NP	.08180	05250	03900	478 6	619 1	1,049 0	2,146.7
NK	08200	05500	.04040	479 8	648 6	1,083 0	2,211 4
N	.08200	05200	04000	479 8	625 0	1,075.9	2,180 7
Cultivation.							
Check	.07110	.05100	.03445	415 9	601 5	926.6	1,941.0
PK	.07485	05000	.03571	436.4	590 4	960 5	1,987.3
NPK	.07800	05290	.04310	456 4	620 8	1,146 6	2,223 8
NP	07900	.05230	.04250	462.2	614 4	1,142.9	2,219 5
NK	.07820	.05500	04350	457 6	648 8	1,170 1	2,276 5
N	.07890	.05200	.04200	461 6	620.2	1,131.9	2,213 7

Table 2 gives the percentage and absolute amounts of nitric nitrogen and of nonnitric nitrogen. The latter values were obtained by difference between the total nitrogen and the nitric nitrogen.

TABLE 2.—*Percentage and absolute amounts of nitric and nonnitric nitrogen in the respective horizons before trees were planted and at the end of the experiment*

Treatment	Nitric nitrogen			Absolute amount nitric nitrogen			
	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Total (0-53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.00170	0.00053	0.00023	9.9	6.3	6.2	22.4
Sod.							
Check	.00069	.00080	.00060	4.0	9.4	16.1	29.5
PK	.00070	.00100	.00066	4.1	11.8	17.7	33.6
NPK	.00220	.00760	.00450	12.9	89.6	121.0	223.5
NP	.00200	.00720	.00500	11.7	84.9	133.5	230.1
NK	.00290	.00900	.00560	16.9	106.1	147.0	270.0
N	.00240	.00820	.00550	14.0	96.7	147.9	258.6
Cultivation							
Check	.00110	.00100	.00065	6.4	11.8	17.5	35.7
PK	.00085	.00120	.00071	3.5	14.1	19.1	36.7
NPK	.00210	.00640	.00410	12.3	72.4	111.0	195.7
NP	.00200	.00610	.00460	11.7	69.6	123.5	204.8
NK	.00270	.00690	.00590	15.8	81.5	158.7	256.0
N	.00290	.00760	.00500	16.9	89.5	136.7	243.1

Treatment	Nonnitric nitrogen			Absolute amount nonnitric nitrogen			
	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Surface (0-7 inches)	Subsurface (7-21 inches)	Subsoil (21-53 inches)	Total (0-53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.0848	0.0495	0.0350	496.2	583.4	940.6	2,020.2
Sod.							
Check	.0815	.0452	.0334	473.9	533.1	898.4	1,905.4
PK	.0813	.0460	.0343	475.7	542.5	923.7	1,941.9
NPK	.0798	.0444	.0345	468.9	523.6	928.0	1,918.5
NP	.0798	.0453	.0340	466.9	534.2	915.5	1,916.6
NK	.0791	.0460	.0348	462.9	542.5	936.0	1,941.4
N	.0796	.0448	.0345	465.8	528.3	928.0	1,922.1
Cultivation							
Check	.0700	.0500	.0338	409.5	589.7	909.1	1,908.3
PK	.0740	.0488	.0350	432.9	576.2	941.4	1,950.5
NPK	.0759	.0465	.0390	444.1	548.4	1,035.6	2,028.1
NP	.0770	.0462	.0379	450.5	544.8	1,019.4	2,014.7
NK	.0755	.0481	.0376	441.8	567.3	1,011.4	2,020.5
N	.0760	.0450	.0370	444.7	530.7	995.2	1,970.6

DISCUSSION OF DATA

TOTAL NITROGEN

The data in table 1 indicate that at the end of the experiment the total nitrogen content of the surface soil in all cylinders under sod was slightly greater than that in cylinders under cultivation, although, as has already been pointed out, the cylinders under cultivation had received 15.9 grams more nitrogen than the corresponding cylinders under sod. The differences between the total nitrogen content of corresponding cylinders under the two systems in the respective horizons are shown in table 3.

The data in table 3 do not take into account the nitrogen removed by the trees and, in sod, by the grass also. The disappearance of nitrogen (as total nitrogen) when the amounts removed by the trees are taken into account is shown in table 4. This disappearance is called by some investigators "the apparent loss of nitrogen."

TABLE 3.—*Difference* [a] *(in grams) between the amounts of total nitrogen present in the 3 horizons of the 2 systems*

Horizon	Check	PK	NPK	NP	NK	N
Surface (0-7 inches)	+62.0	+43.4	+23.4	+16.4	+22.2	+18.2
Subsurface (7-21 inches)	-59.0	-36.1	-7.6	+4.7	0	+4.8
Subsoil (21-53 inches)	-12.1	-19.1	-97.6	-93.9	-87.1	-56.0
Total (0-53 inches)	-9.1	-11.8	-81.8	-72.8	-64.9	-33.0

[a] The sign indicates the amount in grams by which the total nitrogen under sod is greater than (+) or less than (−) under cultivation.

TABLE 4.—*Nitrogen disappearance (grams) calculated on the total nitrogen of the soil in the whole layer (0 to 53 inches) at the end of the experiment*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) Amount N present in soil before experiment	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6
(2) Amount N added in $NaNO_3$ (=465.0 g)+seeds (=2.5 g)	2.5	2.5	467.5	467.5	467.5	467.5
(3) Amount N from (1)+(2)	2,045.1	2,045.1	2,510.1	2,510.1	2,510.1	2,510.1
(4) Amount N found	1,934.9	1,975.5	2,142.0	2,146.7	2,211.4	2,180.7
(5) Loss of N from soil	-110.2	-69.6	-368.1	-363.4	-298.7	-329.4
(6) Total amount N absorbed by trees during growth (tops and roots)	36.5	56.8	201.4	190.4	133.4	124.3
(7) Disappearance of N by leaching and possibly as gaseous N during the 6½ years of the experiment	-73.7	-12.8	-166.7	-173.0	-165.3	-205.1

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) Amount N present in soil before experiment	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6
(2) Amount N added in $NaNO_3$ (=465.0 g)+seeds (=2.5 g)	19.4	19.4	483.4	483.4	483.4	483.4
(3) Amount N from (1)+(2)	2,062.0	2,062.0	2,526.0	2,526.0	2,526.0	2,526.0
(4) Amount N found	1,944.0	1,987.3	2,223.8	2,219.5	2,276.5	2,213.7
(5) Loss of N from soil	-118.0	-74.7	-302.2	-306.5	-249.5	-312.3
(6) Total amount N absorbed by trees during growth (tops and roots)	53.5	63.4	180.9	170.3	131.8	121.3
(7) Disappearance of N by leaching and possibly as gaseous N during the 6½ years of the experiment	-64.5	-11.3	-121.3	-136.2	-117.7	-191.0

Considering the whole depth 0 to 53 inches, the losses from the nitrogen-treated cylinders are greater under sod than under cultivation. The amounts by which the losses (in grams) under the former system exceed those under the latter are: NPK, 45.4; NP, 36.8; NK, 47.6; and N, 14.1 These differences appear to be related to the accretion of nonnitric nitrogen in the subsoil of the cylinders under cultivation. The net result is a gain in nonnitric nitrogen when calculated on the whole depth (0 to 53 inches) in the treated cylinders under cultivation as compared with those under sod. This point is discussed later.

Line 7 of table 4 gives the losses by leaching and possibly as gaseous nitrogen (i.e., the so-called "nitrogen balance") for the whole soil layer (0 to 53 inches). The same data calculated for the surface 0 to 7 inches and subsurface 0 to 21 inches only are shown in table 5.

TABLE 5.—*Nitrogen balance (in grams) calculated to less than full depth*

Horizon	Check	PK	NPK	NP	NK	N
0 to 7 inches.						
Under sod	+5.8	+34.0	−292.4	−304.6	−360.4	−369.5
Under cultivation	−56.1	−25.7	−352.2	−357.0	−400.1	−406.6
0 to 21 inches:						
Under sod	−41.4	−7.4	−268.9	−275.2	−301.5	−334.2
Under cultivation	−44.3	−25.0	−321.1	−332.3	−341.0	−376.1

The nitrogen balance is seen to vary with the depth of soil upon which the calculations are based. In the 0- to 7-inch layer, a nitrogen gain is indicated in the cylinders under sod to which no mineral nitrogen was added. But if the calculations are based on the 0- to 21-inch layer or on the whole depth, 0- to 53-inch layer, losses of nitrogen are definitely established. The larger losses shown in the nitrated cylinders in the 0- to 7-inch and 0- to 21-inch layers as compared with the 0- to 53-inch layer appear to be only an expression of the fact that the quantity of nitrates (nitric nitrogen) becomes greater with depth.

More information with respect to the status of the nitrogen is obtained by considering the nitric and nonnitric fractions of the total nitrogen separately. These are shown in table 2.

NITRIC NITROGEN (NITRATES)

MOBILITY OF ADDED NITRIC NITROGEN

Table 2 shows that the nitric nitrogen calculated on a percentage basis, i.e., the concentration of nitrates, is greater in the subsurface than in either of the other horizons. The absolute amount of nitric nitrogen, however, is greater in the subsoil in all cases. The last application of $NaNO_3$ (167 grams nitrogen) was made in the spring of 1927, 4½ months before the trees were removed. Presumably, therefore, this increased concentration of nitric nitrogen in the subsurface is merely an expression of the movement of nitrogen as nitric nitrogen from this last application, and when taken in conjunction with the data for the nitric nitrogen in the check and PK cylinders, suggests that the greater part of the last application was still in the 7- to 21-inch layer at the conclusion of the experiment.

NITRIC NITROGEN UNDER SOD AND CULTIVATION

It will be recalled that each of the cylinders under cultivation received 15.9 grams more nitrogen in the form of rye cover crop than the cylinders under sod. Now the concentration of nitric nitrogen in the whole depth (0 to 53 inches) of the check cylinder under cultivation is 35.7 grams and that of the cylinder under sod is 29.5 grams as compared with 22.4 grams in the original soil. However, in the cylinders which received mineral nitrogen in addition to that introduced by the green manures (the NPK, NP, NK, and N cylinders) the concentration of nitric nitrogen in the whole depth is much greater in all cases in the cylinders under sod. This may be only another expression of the difference in the status of the soil nitrogen in the three horizons under sod and cultivation previously referred to in the discussion of the disappearance of nitrogen (as total N) by leaching and possibly as gaseous nitrogen. This will be brought out more clearly in the discussion of the nonnitric nitrogen fraction.

AN INVENTORY OF NITRIC NITROGEN

A more complete picture of the status of the nitrates may be obtained from the inventory of nitric nitrogen shown in table 6, in which account has been taken of the nitric nitrogen equivalent to that absorbed by the trees under the different treatments from the added $NaNO_3$.

TABLE 6.—*Inventory of nitric nitrogen (in grains) at end of experiment (0 to 53 inches)*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) N added to each cylinder in the form of $NaNO_3$	0	0	465.5	465.5	465.5	465.5
(2) N absorbed by each tree during growth and also (in sod) by the grass	36.5	56.8	201.4	190.4	133.4	124.3
(3) N absorbed by each tree from added $NaNO_3$	0	0	132.7	132.4	91.9	83.6
(4) Theoretical amount of N expected in soil			332.8	333.1	373.6	381.9
(5) N found	29.5	33.6	223.5	230.1	270.0	258.6
(6) Disappearance of nitric N during the 6½ years of the experiment			109.3	103.0	103.6	123.3

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) N added to each cylinder in the form of $NaNO_3$	0	0	465.5	465.5	465.5	465.5
(2) N absorbed by each tree during growth and also (in sod) by the grass	53.5	63.4	180.9	170.3	131.8	121.3
(3) N absorbed by each tree from added $NaNO_3$	0	0	117.5	93.2	78.3	67.8
(4) Theoretical amount of N expected in soil			348.0	372.3	387.2	397.7
(5) N found	35.7	37.0	195.7	204.8	256.0	243.1
(6) Disappearance of nitric N during the 6½ years of the experiment			152.3	167.5	131.2	154.6

The nitric nitrogen absorbed by the trees (table 6, line 3) was obtained in a manner similar to that described in an earlier paper (*15, pp. 570–573*). The values in line 3 represent the difference between the amount of nitrogen absorbed by a tree which received additions of another element (or other elements) than nitrogen and a tree from which nitrogen was omitted. The values so obtained may not be mathematically exact, inasmuch as the Wirkungswert (effect factor) of an element may not be the same in the presence of another factor or factors as when that factor operates alone. Nevertheless, there are numerous experiments that lend support to Mitscherlich's contention (*7*) that the Wirkungswert of an element may be fairly constant, especially under the controlled conditions of such an experiment as the present one. The method is believed to be sufficiently accurate to bring out more clearly any characteristic differences in the status of the nitric nitrogen of the respective treatments and especially with respect to differences between the two cultural systems. The procedure adopted may be more readily understood from the following algebraical analysis:

Let a = amount of nitrogen present in the soil of each cylinder before the trees were planted.

Let x = amount of nitrogen added to each cylinder in rain and snowfall.
Let y = amount of nitrogen added to each cylinder under cultivation in the form of organic matter (buckwheat and rye).
Let z = the total amount of sodium nitrate added to each of the "nitrated" cylinders.

Now the nitrogen-treated trees have obtained the nitrogen absorbed by them from all of the foregoing sources, and the trees which did not receive mineral nitrogen ($NaNO_3$) additions absorbed nitrogen from all of these sources except z.

For greater simplicity and clarity, let us first of all consider the absorption of nitrogen from only two of the trees on which chemical analyses were made; namely, the NPK and the PK trees, both under the tillage system.

The amount of nitrogen absorbed by the NPK tree during the whole period of its growth will be some fraction of $a+x+y+z$. Let this fraction be designated $k(a+x+y+z)$. Similarly, the amount of nitrogen absorbed by the PK tree during the whole period of its growth will be some fraction of $(a+x+y)$, which will be designated $k'(a+x+y)$.

Let $\frac{1}{s}=k(a+x+y+z)$ (1)

Let $\frac{1}{r}=k'(a+x+y)$ (2)

Then, by subtraction

$\frac{1}{s}-\frac{1}{r}=k(a+x+y+z)-k'(a+x+y)$ (3)

Now, if $k(a+x+y)$ is equal to or very nearly equal to $k'(a+x+y)$,

then, from (3), $\frac{1}{s}-\frac{1}{r}=kz$ (4)

or, expressed in words, the fraction of the nitrogen added to the NPK tree in the form of sodium nitrate is obtained by difference between the total amount of nitrogen absorbed by that tree and that absorbed by the PK tree.

In the present experiment the trees were grown without mineral salt additions for the first 4 years. The factor z then of equation (1) does not enter into the picture during this period.

For the purpose of the present analysis the difference between the quantities $k(a+x+y)$ must be very small as compared with the quantity kz. In further support of this contention may be cited the mathematical analysis given by the writer in an earlier paper (*13*).

The amounts of nitrogen applied as sodium nitrate were 149 g in 1925, 149 g in 1926, and 167 g in 1927, a total of 465 g. A comparison of these quantities of added nitrogen with the quantities actually present (table 6, line 5) shows that all of the "nitrated" cylinders contained at the end of the experiment more than enough nitrates to account for the amount (167 g) added the last year of the experiment, 4½ months before the samples were taken in the fall of 1927, and in addition a considerable portion of the nitrogen added in the second application in the spring of 1926. The total precipitation during the period between one application and the next was: May 18, 1925, to May 2, 1926, 31.8 inches; May 3, 1926, to May 4, 1927, 44 inches; May 5, 1927, to September 20, 1927, 18.6 inches. In addition, 2 inches of artificial watering was applied in May 1926 and 1 inch in August 1927.

The Rothamsted experiments (*9*) on the losses of nitrogen in the drainage waters from a plot of arable land kept free of vegetation since 1870, which received no artificial additions of nitrogen, are frequently cited in support of the view that nitrates are readily leached from soils. At the end of 47 years the amounts of nitric nitrogen found in the drainage waters were equal to the total losses of nitrogen from the soil. The rate of loss was equal to 40 pounds per annum per acre in the earlier years and below 25 pounds per annum per acre in the later years.

In some soil types in Tennessee (*8*), however, when nitrogen was applied as sodium nitrate to lysimeters kept bare of vegetation, the leaching (outgo) of nitrogen in 2½ years was as low as 34 percent. Mooers and MacIntire attribute this relatively small loss to the influence of the heavy clay subsoil into which the nitrate ion passes as magnesium and calcium nitrates through base exchange.[3]

The investigations of De Sornay (*10*) also indicate that nitrates may remain in the soil available to plants for long periods, moving upward or downward according to moisture conditions. The upward capillary attraction was found to be much more rapid than the downward displacement by rain. De Sornay reports that Demolon and Brouet at Aisne found, in an uncropped, light, sandy garden soil, that after a rainfall of 9.8 inches during a period of 2 months more than one half of the added sodium nitrate remained in the first 8 inches.

More recently Ames (*2*) has reported that during the period 1928–30 the nitric nitrogen content of the soil under corn or soybeans never exceeded 50 pounds per acre in the surface 6⅔ inches, but in 1931, after a year of drought, the nitric nitrogen content reached 300 pounds per acre.

The problem is summed up by MacIntire [4] as follows:

> It is exceedingly difficult to make an unqualified statement as to the fate of added nitrogen. This will vary with the soil, alkalinity or acidity, climatic conditions, the amount of added nitrogen, absence or presence of growing plants and the type of these, and periodicity of rainfall, together with the very important fact of depth and type of subsoil.

In the present experiment the significant fact is that the disappearance of nitric nitrogen is greater in all the cylinders under cultivation to which mineral nitrogen was added than under the corresponding cylinders under sod.

NONNITRIC NITROGEN

The nonnitric nitrogen consists of (1) nitrogenous organic material potentially "available" but not yet decomposed; (2) the humus nitrogen characterized by marked stability; (3) the nitrogen synthesized by the micro-organisms; (4) ammonia nitrogen absorbed by the colloidal soil complex.

The apparent gain or loss in integral numbers with respect to the nonnitric nitrogen is given in table 7. The quantities of nitrogen shown in line 4, that is, the amount of nitrogen absorbed by the trees from sources other than the nitrogen added as $NaNO_3$, were obtained by using the quantities of nitrogen absorbed by corresponding treatments to which no nitrogen was added. For example, the amount of nitrogen absorbed by the NPK tree in sod from sources other than that equivalent to the added $NaNO_3$ was obtained by difference between this quantity and that absorbed by the PK tree also growing in sod. For reasons already given these values have no pretention to mathematical exactness. But in the present analysis they serve for all practical purposes to furnish a picture of the changes in nonnitric nitrogen of the original soil as a result of the various treatments under the two culture systems.

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[3] MacIntire, W. H. Private communication.

[4] MacIntire, W. H. See footnote 3.

TABLE 7.—*Gain or loss (grams) of nonnitric nitrogen from whole depth of soil (0 to 53 inches) by the end of the experiment*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) Nonnitric N before experiment	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2
(2) Nonnitric N at end of experiment	1,905.4	1,941.9	1,918.5	1,916.6	1,941.4	1,922.1
(3) Actual loss or gain by soil	−114.8	−78.3	−101.7	−103.6	−78.8	−98.1
(4) N absorbed per tree from sources other than the added $NaNO_3$	36.5	57.0	57.0	49.9	37.4	37.4
(5) Apparent total loss or gain during the 6½ years of the experiment	−78.3	−21.3	−44.7	−53.7	−41.4	−60.7

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) Nonnitric N before experiment	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2
(2) Nonnitric N at end of experiment	1,908.3	1,950.5	2,028.1	2,014.7	2,020.5	1,970.6
(3) Actual loss or gain by soil	−111.9	−69.7	+7.9	−5.5	+.3	−49.6
(4) N absorbed per tree from sources other than the added $NaNO_3$	53.5	63.4	63.4	77.1	53.5	53.5
(5) Apparent total loss or gain during the 6½ years of the experiment.	−58.4	−6.3	+71.3	+71.6	+53.8	+3.9

In both culture systems there is a disappearance of nonnitric nitrogen in the cylinders which received no mineral nitrogen additions (check and PK cylinders), the loss being greater under sod. But, whereas relatively large accretions of nonnitric nitrogen have occurred in the NPK, NP, NK, and N cylinders under cultivation, losses of nonnitric nitrogen have occurred from the corresponding cylinders under sod. These differences are much greater than can be accounted for by sampling or analytical errors. Although uniformity with respect to content of nitrogen, phosphorus, and potassium was established in the cylinders before the experiment began, there may have existed differences in respect to the physical condition that would preclude a definite and unqualified interpretation of the differences existing with respect to the condition of the nitrogen under sod and cultivation. Three explanations may be advanced:

(1) Assimilation of nitrogen added as $NaNO_3$ by micro-organisms. But the difficulty lies in explaining why assimilation should have occurred in the cylinders under cultivation and not in those under sod. Carbon dioxide accumulation under grass (*12*) may be the differential factor. Some nitrogen would appear to have been brought up and immobilized in the surface soil by the grass roots in the sod system, inasmuch as the quantity of nitrogen (as total and nonnitric nitrogen)

of the surface soil is higher in all cases under grass than under cultivation. But the results for the whole depth (0 to 53 inches) show that this explanation is insufficient to account for the entire difference in the amount of nitrogen in corresponding cylinders in the two systems.

(2) The peptization of nitrogenous organic material by $NaNO_3$ in the first horizon and subsequent leaching. Hardpan formation was particularly noticeable in the nitrogen-treated cylinders under cultivation. Cracks and fissures, therefore, may have assisted the downward movement.

(3) The greater root system under cultivation. Except in one tree (NPK) the root systems were larger in the trees grown under cultivation. The weights of the root systems are given in table 8.

TABLE 8.—*Weights in grams of the respective root systems in soils in sod and under cultivation*

Condition	Check	PK	NPK	NP	NK	N
Sod	9, 950	8, 325	13, 040	12, 730	11, 090	10, 366
Cultivation	10, 870	10, 695	12, 395	13, 075	11, 700	12, 275

The higher nitrogen content of the soil under cultivation may have arisen from sloughed-off portions of fibrous roots that might have been incorporated in the soil in the process of preparation for analysis.

The extensive literature of the problem of the mineralization of nitrogen in the soil has been reviewed by Lemoigne (*5*), Lyon (*6*), and Waksman (*17*). The results presented in this paper suggest the desirability of further investigation of the problem.

SUMMARY

The distribution of total nitrogen and also of the nitric and nonnitric fractions in three horizons, viz, 0 to 7 inches, 7 to 21 inches, and 21 to 53 inches, of a Hagerstown clay loam soil contained in cylinders planted to apple trees and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate are given in percentage and in absolute amounts.

In all treatments the total nitrogen of the surface soil under sod was somewhat greater than under cultivation. In the subsurface the differences in total nitrogen were small except in the check cylinders under sod, in which it was less than under tillage. In all treatments the total nitrogen of the subsoil was greater in the cylinders under cultivation than in the corresponding cylinders under sod. For the whole depth (0 to 53 inches), the total nitrogen at the end of the experiment was greater in all cylinders under cultivation than in those under sod.

The disappearance of nitrogen (as total nitrogen) by leaching, and possibly as gaseous nitrogen, was greater under sod than under cultivation in all cases.

The movement of nitric nitrogen is discussed. It is concluded that leaching of nitrates from this heavy soil was not very rapid.

The disappearance of nitric nitrogen, when account has been taken of the nitric nitrogen absorbed by the trees equivalent to that added as $NaNO_3$, was greater in all nitrated cylinders under cultivation than

in corresponding cylinders under sod. This difference is account for by an accretion of nitrogen as nonnitric nitrogen in the subs under cultivation but not in that under sod.

Results with respect to nitrogen gains or losses based on the soil t a depth of 53 inches are very different from those based on the 0- t 7-inch or 0- to 21-inch depths.

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LIFE HISTORY OF THE HAIRY-ROOT ORGANISM IN RELATION TO ITS PATHOGENESIS ON NURSERY APPLE TREES[1]

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INTRODUCTION

In studies of infectious hairy root carried on during the last 4 years, special consideration has been given to the life history of the causal organism in relation to its pathogenesis on nursery apple trees. This disease has been so prevalent on grafted apple trees in the nursery that it has become of considerable economic importance. The causal organism, *Phytomonas rhizogenes* Riker et al., has recently been differentiated by Riker and his associates (*26*)[3] as a new species distinct from that causing crown gall, *P. tumefaciens* (Smith and Town.) Bergey et al. Previously it had been considered an apple strain of the crown-gall organism by Smith et al. (*36*) and more completely developed as such by Siegler (*33, 34*).

The name "hairy root" appears to have been first introduced into literature by Stewart, Rolfs, and Hall (*37*). Following the earlier work of Hedgcock (*11*) on the identity of the complex of malformations occurring on apple, a number of well-known papers appeared. Recently a number of diseases have been separated from this complex on the basis of cause, viz, (1) infectious hairy root, (2) crown gall, (3) wound overgrowth, and (4) nonparasitic hairy root. Of these, infectious hairy root is now perhaps the most important from the economic standpoint.

The host range of the hairy-root organism is little understood. Up to the present time the writer has found it reported under natural conditions only on apple. However, cross-inoculation studies by various workers, including Smith et al. (*36*), Riker et al. (*25*), Brown (*6*), Banfield (*3*), and Riker et al. (*26*), have demonstrated the pathogenicity of these bacteria on such plants as sugar beet (*Beta vulgaris* L.) quince (*Cydonia oblonga* Mill.), rose (*Rosa setigera* Michx.), honeysuckle (*Lonicera morrowi* Gray), Paris daisy (*Chrysanthemum frutescens* L.), balsam (*Impatiens balsamina* L.), bryophyllum (*Bryophyllum pinnatum* Kurz), red raspberry (*Rubus idaeus* L.), bean (*Phaseolus vulgaris* L.), and sedum (*Sedum spectabile* Bor.).

Hairy root appears to be widely distributed. Hedgcock (*11*), who reported crown gall from all the States of the United States except

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[3] Reference is made by number (italic) to Literature Cited, p. 883.

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Nevada, stated that "the forms of the disease known as hairy root have been found as widely disseminated as crown gall on apple trees in nurseries and orchards in the United States." He also reported forms of hairy root from Germany, Netherlands, France, Canada, and New Zealand. Doidge (*8*) recorded the presence of the disease in South Africa, and Noble (*18*) in Australia. The disease seems widely disseminated, but the difficulties of diagnosis raise a question as to the accuracy of some of the reports.

The economic importance of infectious hairy root, although considerable, is hard to estimate since no information is available as to how much of the loss due to malformations may be attributed to this disease. Studies in which the writer has participated for more than 4 years, in nurseries from Wisconsin to Oklahoma, reveal that while other difficulties in this complex have been largely eliminated hairy root still remains a factor of considerable importance in certain places.

Control measures at the present time are only partially satisfactory. Von Schrenk and Hedgcock (*39*) laid the foundation for the most successful of later attempts to control the various malformations at the unions of piece-root grafts when they noted that these enlargements usually appeared at the graft union, that using root and scion pieces of nearly the same diameter reduced overgrowths, and that wrapping the unions with certain materials such as cloth and rubber considerably increased the percentage of smooth trees over those wrapped with other materials. Since the publication of their report many control measures have been suggested by different workers, as, for example, Melhus and Maney (*15*), Wormald and Grubb (*41*), Riker and Keitt (*30*), Waite and Siegler (*40*), Melhus, Muncie, and Fisk (*16*), and Maney and Pickett (*14*). Additional studies by Riker and his associates (*21, 24, 27, 31*) have repeatedly demonstrated the value of nurseryman's tape, a special kind of adhesive plaster. Since the discovery that this tape prevents a large percentage of union malformations without producing any ill effects, it has come into common use. Among the nurserymen there is a feeling that the saving in handling the grafts bound with this wrapper more than pays for its extra cost. In addition, there is a considerable increase in the number of clean trees. The use of nurseryman's tape has eliminated on an average more than half of the various outgrowths at the union. Those that remain are mostly hairy-root.

The persistence of malformations, chiefly of the hairy-root type, caused by *Phytomonas rhizogenes*, appeared to warrant a study of the life history of the causal organism in relation to pathogenesis. It was hoped that such a study would not only increase the available information on the fundamental activity of this organism but would also define critical points at which the application of control measures might be more effective.

IDENTITY OF HAIRY ROOT

Before studying the pathogenic life history of infectious hairy root it seemed desirable to make a reexamination of the identity of hairy root as contrasted with crown gall, wound overgrowth, and other malformations occurring on the underground parts of nursery apple trees.

The isolation of the hairy-root organism was attempted from a variety of malformations on Wealthy apple trees collected at random at digging time from the experimental plots at Madison, Wis., and Topeka, Kans. It was found from the outward appearance and interior structure that these overgrowths could be roughly classified into three groups: (1) Convoluted, with roots, characterized by a hard vascular interior and a soft exterior layer of variable thickness that turned brown rapidly when exposed to the air; (2) convoluted, without roots, identical with the foregoing except for the absence of roots; and (3) undulated, without roots and, unlike the other enlargements, chiefly made up of whitish vascular cortex. The enlargements differed considerably in appearance and in the number of emerging roots, making them difficult to classify. Nevertheless it seemed important to attempt isolations from such specimens.

The method of isolation was as follows: From a specimen of each type of overgrowth several cubes of overgrowth tissue, approximately 3 mm on a side, were removed from the soft tissue, if present, under aseptic conditions and dropped into three Petri dishes each containing 1 cc of sterile distilled water. A sterilized scalpel was used to macerate the tissue in the water. After an interval of about 10 minutes dilutions were made from each plate to three successive Petri dishes. Patel's (*19*) bile agar was then added. The poured plates were incubated at 28° C. and examined after 1 week. The identity of the bacteria that appeared was determined after inoculations on sedum and apple and after cultural examination, as suggested by A. J. Riker, on sodium selenite agar. A summary of these studies is given in table 1, showing that a majority of the convoluted enlargements with roots were infectious hairy root and that a considerable part of the convoluted enlargements without roots were also hairy root. The undulated enlargements did not yield the infectious agent and in all probability they were nonparasitic overgrowths. Since the experiment described below showed the isolation technic to be reasonably accurate, it appears that many of the specimens contained tissue not primarily stimulated by the hairy-root bacteria. Perhaps the reaction induced by the hairy-root tissue may have stimulated nonparasitic growth in some cases. These results appear to be in conformity with those of Riker et al. (*26*).

TABLE 1.—*Summary of isolation trials from different kinds of enlargements*

Surface character of enlargement [a]	Isolation trials	Enlargements yielding—		
		Phytomonas rhizogenes	Nonpathogenic bacteria	No bacteria
	Number	*Percent*	*Percent*	*Percent*
Convoluted, with roots	114	60	24	16
Convoluted, without roots	13	39	54	7
Undulated, without roots	25	0	32	68

[a] Characterization of these enlargements is given in the text

The hairy-root organism was reisolated from hairy-root enlargements induced on nursery apple trees by inoculating the stems below ground. These induced enlargements, ranging in age from 0 to 24 weeks, covered the complete range of symptoms. The same isolation technic was employed as in previous experiments. The results are summarized in table 2. All stages of the disease yielded the causal organism. Out of 129 trials, the hairy-root organism was recovered in 87 percent, indicating that the causal organism is usually associated with the symptoms of the disease. Nonpathogenic bacteria were recovered in 13 percent of the trials. None of the enlargements was found to be free from bacteria.

TABLE 2.—*Summary of reisolation trials from infectious hairy-root enlargements of different ages, induced by inoculations on nursery apple trees*

Year	Period following inoculations	Isolation trials	Enlargements yielding—	
			Phytomonas rhizogenes	Nonpathogenic bacteria
	Weeks	*Number*	*Percent*	*Percent*
1929	0-4	27	93	7
	5-8	20	60	40
	9-12	8	50	50
1930	0-4	18	100	0
	5-8	14	80	20
	9-12	10	100	0
	13-16	14	100	0
	17-20	12	100	0
	21-24	6	100	0
Total		129	87	13

The roots of infectious hairy root have not been found to contain the hairy-root bacteria. Only negative results were secured from attempts to isolate the organism from the tissues of 37 fleshy hairy roots more than one fourth of an inch long, which had emerged from the basal enlargements. This finding confirms the work of Smith et al. (*36*) and Riker et al. (*26*), who found the bacteria to be present only in the basal enlargements.

Isolation trials from crown gall and wound overgrowth induced by inoculations and wire girdles, respectively, appear to establish the identity of these malformations. A series of reisolation studies somewhat less extensive than those conducted on hairy root were made on induced crown gall and wound overgrowth. In 26 trials the crown-gall organism was found constantly associated with the crown-gall symptoms in all stages from 0 to 12 weeks. As no pathogenic bacteria were secured in the 10 trials from wound overgrowths, these malformations apparently are distinct from each other and from hairy root. These results are in accord with those of Riker and Keitt (*30*), Muncie (*17*), Siegler (*33*), Brown (*6*), and others, and, in the writer's opinion, establish the identity of the hairy-root disease.

ENTRANCE OF ORGANISM INTO HOST

MATERIALS AND METHODS

In most of the field studies the Yellow Transparent variety of apple was selected as host plant because it was considered relatively susceptible to infectious hairy root and because it was grown in sufficient numbers for the studies projected. Both first- and second-year trees were utilized. These trees were produced from piece-root grafts made from scions and roots grown in Kansas.

The principal plants used in the greenhouse were Paris daisy, bean, sugar beet, sedum, and apple. In a series of pathogenicity studies sedum and sugar beet were found to be the best plants tried for greenhouse studies. In these studies an average of 54 inoculations were made for each kind of plant. The following percentages of wounds in the respective hosts showed hairy-root symptoms 2 months after inoculation: Balsam, 36; bean, 58; bryophyllum, 57; Paris daisy (single yellow), 66; Paris daisy (single white), 36; sedum, 100; sugar beet, 100; begonia, 0; and tomato, 0. A similar study of 240 wound inoculations on the Fameuse variety of apple grown in pots showed 52 percent infected.

The bacterial culture chiefly employed was the progeny of a single cell, strain C-1 (fig. 1), of the hairy-root organism, a detailed description of which is given by Wright, Hendrickson, and Riker (*42*). At 3-month intervals this strain was checked for comparative pathogenicity against four sister single-cell strains, C-10, C-11, C-12, and C-13. Three-day-old transfers grown on potato-mannite agar gave abundant growth and were used for inoculation.

The method of inoculation on herbaceous plants was by needle puncture. The method of inoculation on apple grafts, unless otherwise noted, was as follows: On one side of the row a trench was made in the soil to the depth of the union, about 3 inches away from the trees to avoid injuring them. By means of a dibble the soil was removed from around the individual trees, and the stems were wiped free from soil. Drops of a subculture of the bacteria were then applied with a cotton swab to the stem surface, usually in five places spaced about 1 inch apart. With a scalpel, held at an angle, two thrusts were made through the drops of culture deep into the stem. During the 1930 season, strips of adhesive tape were applied over the wounds to reduce chance contamination of the controls from the soil and to keep out insects. Promptly after inoculation the soil was thrown back into the trench and was hilled up several inches so as to bring it about 2 inches above the topmost wound.

WOUNDS AS INFECTION COURTS

The entrance of bacteria into the host has appeared to be a critical stage in the life history of the hairy-root organism; consequently a series of studies was undertaken to clarify this point.

As the bacteria seemed to enter the host plant only through wounds, the necessity for wounds as points of infection was tested in the following preliminary experiments. The hairy-root organism was washed over the surface of 20 Paris daisy plants. Ten of these were wounded with needle punctures in five places each, and the others were held without injury as controls. After 2 months of incubation all the unwounded plants were free from disease, whereas 41 out of 50, or 82 percent of the places wounded, showed hairy-root symptoms. Two repetitions of this experiment gave similar results. An experiment on sugar beets, in which the same number of plants were used and the same number of wounds were made, resulted in the unwounded plants remaining healthy and all 50, or 100 percent, of the wounded plants becoming infected. In two similar experiments on bean, 62 and 34 percent, respectively, of the places wounded became infected, but none of the unwounded plants showed symptoms of disease. In an experiment on the Yellow Transparent variety of apple the bacteria were washed over 20 stems below ground. Ten of these were wounded in five places each with scalpel cuts. Two months later the unwounded stems were healthy and 28 of the 50 places wounded, or 56 percent, showed symptoms of the disease. These results are in accord with those obtained by Smith et al. (*36*), Riker (*21*), and Muncie (*17*), in work with the crown-gall organism. From these studies it appeared that infection occurred only through wounds. Attention was next directed to the various kinds of wounds that might be encountered.

Infection followed the introduction of the bacteria into the tissue. This was determined by several tests. Drops of a fresh culture of the hairy-root organism from a cotton swab were placed in five different places on the stems of 10 Paris daisy plants. The bacteria were carried into the tissues by thrusting a needle through the bacterial masses and then entirely through the stems several times. After 2 months of incubation, 38 of the 50 places wounded, or 76 percent, showed the disease symptoms. A repetition of this experiment gave disease reactions for 28, or 56 percent, of the places wounded. Similar studies in which the same number of wounds were made on bean, sugar beet, sedum, and apple, gave, respectively, 34, 100, 100, and 48 percent of infections after incubation periods of about 2 months. This type of inoculation technic was very effective in producing infection, and by means of a scalpel instead of a needle it was largely used in the field experiments on apple trees.

Infection followed the placing of bacteria on the surface of wounds. This was determined by a series of tests. In a preliminary experiment a set of 10 Paris daisy plants were wounded by passing a needle through the stems in each of 5 places. The bacteria were then applied to the wound surfaces in the usual manner with a cotton swab. After 2 months of incubation, 36 of the places wounded, or 72 percent, showed symptoms of the disease. As shown later, the results of the infection-court studies, in which this inoculation technic was used, confirm the results of the Paris daisy experiment. These results demonstrate that infection takes place whether the bacteria are applied to the stem before or after the wounds are made and are in accord with those of Riker (*21*) in experiments with the crown-gall organism.

Because of the importance of wounds in bringing about infection, grafting time is an important period for the infection of piece-root-grafted apple trees. Siegler and Piper (*35*) discuss this point. Whether the bacteria commonly do gain entrance at this time is of such importance that it is discussed in a separate paper, on studies of the seasonal development of the disease, by Riker and Hildebrand (*28*). The reactions at the graft union present so many complications that certain phases of this difficult problem have been simplified in the present work through studies of wounds on the scions of actively growing trees.

INFLUENCE OF TYPE OF WOUND

In a series of trials the type of wound apparently made no difference in the kind of overgrowths but did influence somewhat their rate of development and the percentage of wounds that became infected. A preliminary study on a comparison of needle-puncture and scalpel-cut wounds was made on underground stems of first-year apple trees to test their influence in producing infection. Five needle-puncture wounds were made in each of 10 different trees. An equal number of scalpel cuts were made in 10 trees. The procedure was to push the scalpel or needle deep into the stem, and to smear the hairy-root bacteria promptly over the wound with a cotton swab. Host injury was obviously greater from the scalpel cuts, and after an incubation period of 2 months infection seemed to be more pronounced from the scalpel injuries. Subsequently however, this difference gradually diminished. In this experiment all the injured trees became infected. The percentages of infection resulting from needle-puncture and scalpel-cut wounds were 38 and 62, respectively. In the percentage of wounds infected there was a discrepancy between the two types of wounds, and this required further consideration.

A more extensive wound-type study was made to determine whether the hairy-root bacteria could enter apple stems through different types of injuries. Three types of wounds were employed, as follows: (1) Scalpel cut, which was made by pushing a scalpel deep into the stem at an angle; (2) bruise, which was made to resemble cultivation injury by striking the stem with a hammer; and (3) needle puncture, which was made by thrusting a needle deep into the center of the stem. The effects of these injuries were studied on first- and second-year trees. Each type of wound was made in 5 places on the stem below ground on 13 trees of both ages. In each case the hairy-root bacteria were promptly applied to the surface of the wounds on 10 trees. The wounds on 3 trees were left untreated, as controls. Ten weeks later the results were taken. Because of the similarity of reactions on the first- and second-year trees, they are summarized together. For the respective types of injury the percentages of wounds becoming infected were as follows: Scalpel cut, 71; bruise, 66; and needle puncture, 41. Of the control wounds 5 percent of both the scalpel and bruise injuries became infected. The infection of the control wounds might be attributed to some soil vector, such as insects, which had free access to the wounded places. From this study it is apparent that all the different types of injuries served as infection courts. Moreover, the symptoms produced by the infection of the different wounds were practically identical except for those in the needle-puncture injuries, which differed from the others in having (1) a slightly longer incubation period, (2) a somewhat lower percentage of infections, and (3) smaller reactions.

A repetition during 1931 of the experiment testing the three types of wounds (scalpel cut, bruise, and needle puncture) on second-year trees gave results similar to those just described. Strips of adhesive tape were placed over all the places wounded as a protection against the possible interference of soil fauna. Scalpel-cut and bruise wounds again were the most effective infection courts for the hairy-root bacteria, as 82 and 66 percent, respectively, of these wounds became infected. Thirty-six percent of the needle-puncture wounds became infected. All the control wounds were negative. The slightly longer incubation periods for smaller wounds suggested the need for further study of the size of wound in relation to the development of the disease.

Extremely shallow wounds were found to be poor infection courts. In 1930 two types of wounds were tested, surface and scalpel cut. The surface injury consisted of a very shallow scraping of the stem to expose the outermost cortex layers to the bacteria. The scalpel cuts were made as before. Each type of injury was made in 5 different places on each of 40 trees. The wounds on 30 of the trees were inoculated with the bacteria, those on 10 trees being left as controls. All the wounds were covered with adhesive tape. After an incubation period of 10 weeks, 65 percent of the scalpel wounds and only 3 percent of the surface wounds had become infected. An identical experiment repeated in 1931 gave confirmatory results, as 82 and 4 percent, respectively, of the two types of injuries became infected. These results show that shallow injuries to the stems of young apple trees are relatively poor infection courts and that, within limits, the depth as well as the size of wounds is a factor in the amount of hairy-root infection.

LENGTH OF TIME WOUNDS REMAIN OPEN TO INFECTION

The length of time that wounds remain open to infection was next considered. Preliminary studies were made on Paris daisy, sedum, and bean plants in the greenhouse. The temperature of the greenhouse was approximately 22° C. and the relative humidity about 70 percent. In the first series 5 needle-puncture wounds were made in the stems of 22 Paris daisy plants above, at, and below the ground level. At intervals of 1 hour and 1, 2, 3, 4, 5, 6, 7, 8, 16, and 32 days after wounding, a water suspension of a culture of the hairy-root bacteria was applied to the wounds of two plants with a cotton swab. The results were taken after an incubation period of 2 months. It was found that, in this trial in which the plants were kept on an open greenhouse bench, wounds older than 3 days did not become infected. This indicated that wounds 4 days old had formed a barrier sufficient to keep out the organism. Repetitions of the experiment on Paris daisy, bean, and sedum showed that for 3, 2, and 4 days, respectively, the wounds remained open for infection under greenhouse conditions.

These studies were then extended to include the effect of humidity on the time that infection courts remain open in Paris daisy and bean plants. The same time intervals and number of wounds were employed as in the previous tests. Exposure for 2 days before wounding to a relative humidity of approximately 90 percent at the usual greenhouse temperature of about 22° C. caused the wounds to stay open longer for infection. The period was extended to 5 days for both Paris daisy and bean. In a similar experiment, with the same preliminary treatment, on Paris daisy plants held at the same humidity for 9 days after wounding, the period that wounds remained open for infection was increased to 6 days. These results show that wounds on plants in the greenhouse are ordinarily open to infection for only a few days after they have been made and that raising the relative humidity lengthens this period.

The length of time that wounds on apple remained open for infection was approximately 2 days. Field studies on actively growing apple trees in the nursery were made during the seasons of 1929 and 1930. In 1929 three series of infection-court trials were made, starting, respectively, on May 9, June 7, and July 3. In each series, on the day of starting, 5 wounds were made on the underground stems of each of 4 first-year trees for each interval. The intervals tested were 1 hour and 1, 2, 4, 8, 16, 32, and 64 days. Four wounded trees were kept as controls. At the stated time after wounding, a fresh culture of the hairy-root bacteria was applied to the wounds with a cotton swab. At the close of the season results showed that the wounds on these trees remained open infection courts for no longer than 2 days. These data corresponded closely with those for greenhouse plants. The exact time periods for May, June, and July were, respectively, 2, 2, and 1 days. In this experiment the time of year appeared to influence the results slightly. An identical experiment was conducted on second-year trees with almost the same results. However, in this case, in the June series, 2, or 10 percent, of the control wounds and 5, or 7 percent, of the wounds inoculated later than 4 days after wounding became infected. These results caused the writer to suspect the interference of insects.

Similar studies in 1930 consisted of five series, starting, respectively, on May 12, June 1, July 1, August 1, and September 1. The procedure of the previous season was followed throughout except that the unnecessary 32- and 64-day intervals were discarded. Both first- and second-year trees were used. To prevent possible interference from hairy-root bacteria or insects that might be present in the soil, adhesive tape was placed over the wounds promptly after they were made. The results (table 3) are in accord with those of the season before on the first-year trees, and fix the time that wounds ordinarily remained open for infection at approximately 4 days for the month of May and 2 days for the other months. Since the application of the adhesive tape over all the wounds the second season eliminated chance infections other than those shown in the table, it appears reasonable to assume that the tape was a barrier to some wound-producing element in the soil environment. The fact that the weather was more favorable for host growth during May 1929 than during May 1930 would seem to account for the discrepancy of 2 days in the length of time the wounds remained open for infection. Figure 1, *B*, illustrates the typical hairy-root infections for the May series, when the plants were 24 weeks old.

TABLE 3.—*Length of time at different periods in the growing season of 1930 that wounds on 1- and 2-year-old apple trees remained open infection courts for the hairy-root organism* [a]

Period between wounding and applying bacteria	Wounds on trees of indicated age infected during—									
	May		June		July		August		September	
	1 year	2 years	1 year	2 years	1 year	2 years	1 year	2 years	1 year	2 years
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1 hour	15	18	17	16	16	15	20	18	12	12
1 day	16	16	8	16	12	13	15	9	8	9
2 days	16	16	4	2	11	10	12	10	4	7
4 days	9	9	0	0	0	1	0	0	0	0
8 days	0	0	0	0	0	0	0	0	0	0
16 days	0	0	0	0	0	0	0	0	0	0

[a] Wounds were made on May 12, June 1, July 1, Aug. 1, and Sept. 1. 20 wounds were made in each trial. Observations were made on Nov. 10.

The studies on the length of time that wounds remain open infection courts both furnish support to and receive support from similar studies on whether callus may be ordinarily an infection court. Callus was found ordinarily to be a barrier against the invasion of the hairy-root organism. Riker and Keitt (*30*) earlier reported that callus formed on apple grafts was not commonly an open infection

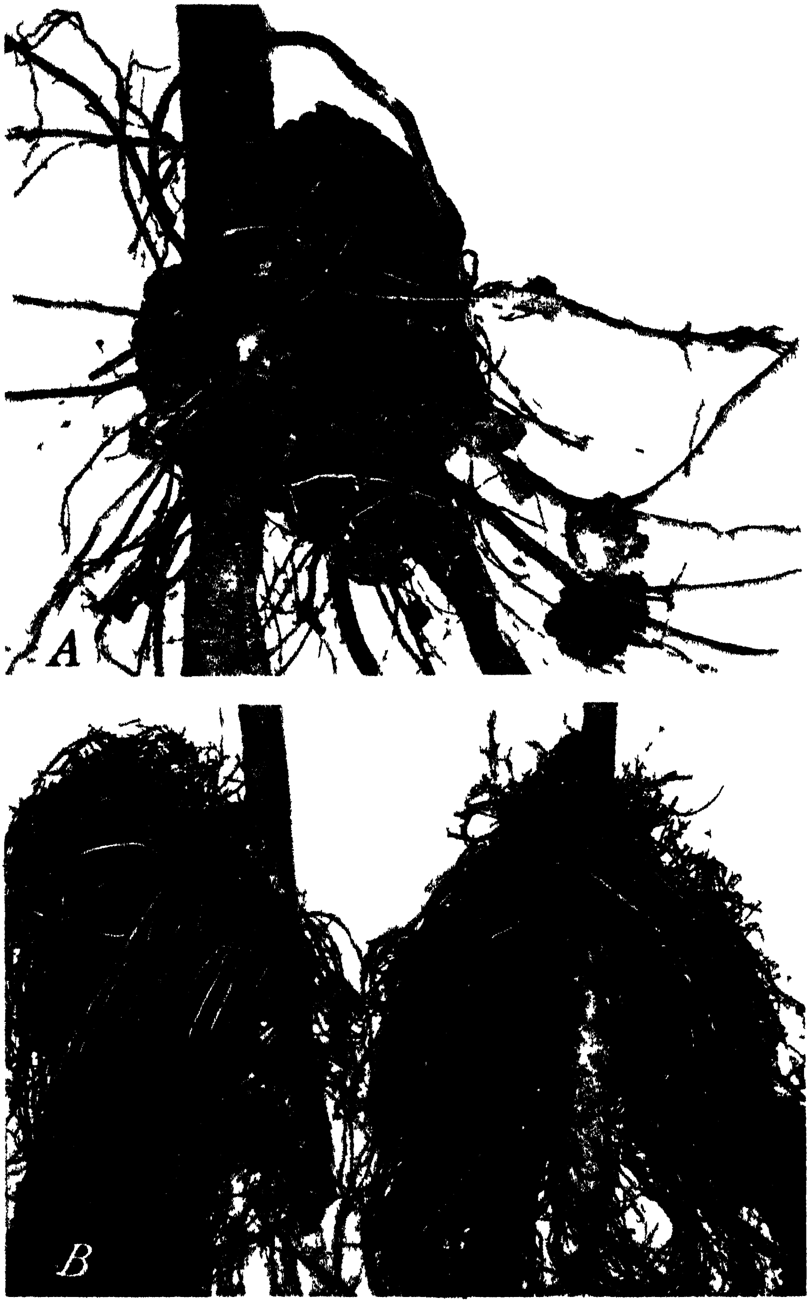

FIGURE 1 *A*, Hairy root overgrowths on the lateral roots of a tree infected on the main stem These lateral overgrowths which yielded the hairy root bacteria, presumably followed insect wounds × $\frac{4}{3}$ *B*, Hairy root infections 24 weeks after inoculation The bacteria were applied to the specimen on the left 1 hour after the wounds were made and to the specimen on the right 1 day after the wounds were made × ½

court for the crown-gall organism. Siegler (*34*) stated that "the graft union after callus has formed is an infection court to a limited extent for the apple organism." Siegler and Piper (*35*) reported a confirmation of Siegler's earlier results, stating that "the grafts are most susceptible at the time they are made and become increasingly resistant with the progression of callus formation."

CALLUS AS A BARRIER TO INFECTION

The infection of callus was studied on apple trees in the nursery. Callus formation was stimulated by wounding the stem, and its reaction toward the application of the bacteria was tested in the soil under natural conditions. Callus of different ages was stimulated by making five wounds below ground on each of eight trees weekly for 10 weeks. On the last date of wounding the bacteria were applied to the wounded surfaces of one half the number of trees. Examination 2 months after inoculation revealed that only those trees wounded on the date of inoculation had become infected. Of these, 11 out of 20, or 55 percent, showed the disease symptoms. The entry of the bacteria in this case had been accomplished before callus had had time to form. Comparable results were obtained in a study by Riker, Hildebrand, and Ivanoff (*29*), similar to that just described except that the callus was stimulated above ground in glass cylinders under approximately aseptic conditions. Similar studies, except for minor changes, were made in 1930. The time, originally 10 weeks, was extended to 12 weeks. Strips of adhesive tape were applied over the wounds to exclude possible soil bacteria and insects. Otherwise the procedure was the same. Again wound callus more than 1 week old did not become infected. Nine out of the 20 wounds inoculated on the day of wounding, or 45 percent, showed symptoms of the disease before callus had had time to form. The results of the season before were verified.

Further studies of callus as an infection court were made in 1931. In one experiment several turns of wire were wrapped around the underground stems of eight trees at weekly intervals for 12 weeks. One week later for each interval, the wrapped portions of the stems of four of the trees were smeared with the bacteria. The other four trees were left untreated, as controls. Observations were made 9 weeks after the application of the bacteria. Although disease symptoms appeared in callus developments of all 12 ages, in 10 percent of the inoculated trees and in 8 percent of the control trees, this study seemed to demonstrate that callus is not commonly an infection court. The fact that a white grub was observed feeding on one of the overgrowths which later showed infection indicates that the chance infections encountered may have been due to insect injuries.

In another experiment callus was stimulated by making a slanting upward cut with a scalpel into the underground stems. To prevent reunion of the several tissues a strip of adhesive tape was inserted in each cut. In all other particulars this experiment was identical with the preceding one. When the observations were made, it was found that 12 percent of the inoculated trees were infected and that the control trees were not infected. These results appear to substantiate those just reported in showing that unwounded callus does not ordinarily serve as an infection court.[4]

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[4] Illustrations of these callus developments will be found in a paper by Riker and Hildebrand (*28*).

Since callus is very easily injured, a study was made of the susceptibility to hairy-root infection of callus that had received small wounds. Shallow wounding did not commonly, if at all, permit the entry of the hairy-root organism. Using the technic previously described, the writer stimulated callus by wounding under three conditions: (1) On underground stems exposed to the soil; (2) on underground stems protected by adhesive tape; and (3) on aerial stems in glass cylinders (*29*) protected from outside contamination. At 4 biweekly intervals 5 wounds were made in each of 2 plants for each of the 3 conditions named above. One week after the last wound was made the callus developments of the various ages were lightly pricked and scratched so as to limit the injury to the outer few layers of callus cells. The bacteria were then smeared over the injured surfaces. After an incubation period of 8 weeks only one of the wounds, and that in a 1-week-old callus, was observed to be infected. In this case it appeared that, after the injury was made, insufficient callus remained over the susceptible host tissues to be a barrier to the organism. Parallel to this an equal number of wound inoculations were made which resulted in the development of the disease. Repetition of one part of this experiment, namely, that on the underground stems protected by adhesive tape, gave the same results the next season. These results indicate that shallow wounds in callus do not ordinarily bring about infection, which is in accord with the results obtained when shallow wounds were made in apple stems.

INFLUENCE OF SOIL INSECTS

Wounds produced by insects should be considered as a possible factor in infection by the hairy-root organism in the nursery. Root-chewing arthropods have been found by Banfield (*4*) associated with the occurrence of crown gall on raspberry. He showed that healthy raspberry plants grown in soil which had been inoculated with the crown-gall organism but which was free from insects became diseased only when white grubs were introduced into the controlled environment. Consequently it seemed desirable to examine the possible relation of insects to hairy-root infection.

Root-feeding insects have been observed during three growing seasons in the soil about nursery apple trees. Preliminary to a more detailed study (*28*) of seasonal development of diseases, including hairy root, a survey was made of the insects commonly present around the graft unions in the soil. From the survey it was determined that root-feeding insects, especially white grubs (Phyllophaga) and wireworms (Elateridae), were commonly present in the nursery soil during the growing season, from May to October.

Insects eating both hairy-root and healthy tissue have frequently been seen during the observations of diseased and healthy trees. These observations were made in the course of studies which required the examination of several hundred young apple trees each week. On several occasions during the growing season, from May to October, white grubs were observed feeding upon hairy-root overgrowth tissue and other underground parts, such as stem tissue, root tissue, and both infectious and noninfectious hairy roots. Observations in 1931 served to support the findings of the two previous seasons as to the activity of this insect and pointed to it as an important agent in producing wounds that might lead to the hairy-root disease if the causal bacteria were present.

Injuries similar to those produced by white grubs in Kansas have been observed in Wisconsin, Iowa, Missouri, Nebraska, and Oklahoma. Wireworms also were occasionally observed burrowing into callus and overgrowth tissues, especially in the earlier stages. Small fungus-gnat larvae (Mycetophilidae) were found to frequent some of the enlargements in the crevices and sometimes the tissues. On three occasions white ants were found making trenches in the main root or in the large branch roots, sometimes for the greater portion of their length. Twice microscopic examination of the surface of five young overgrowths revealed the presence of nematodes. These are but some of the instances of interference by soil fauna with the underground plant parts as observed in the nursery during the growing season.

Over a period of 4 years from one to several specimens of infected lateral roots were observed in positions that could have been reached only by insects. Riker and Hildebrand (*28*) in their studies found from 0 to 0.6 percent of the lateral roots infected. Hairy-root bacteria were isolated from the small overgrowths and from the main overgrowth in the specimen illustrated in figure 1, *A*, proving their infectious nature.

The development of new infections during both the first and the second growing season in Kansas was most easily explained as due to the agency of insects. As stated earlier, wounds appear to be necessary for the entrance of the bacteria. To account for the occurrence of new infections over so long a period, some wound-producing agency must, therefore, have been almost constantly present in the soil around the graft unions. Two examples may be cited. In a block of 240 trees, 39 percent were found infected at the end of the first growing season and 59 percent at the close of the second growing season. A summary of the studies over a period of 4 years, in which about 7,000 trees were examined, showed at the close of the second season an increase in disease development of 13 percent over the first season. Further evidence on this point will be found in the paper on seasonal development by Riker and Hildebrand (*28*).

Several experiments were suggested by the observations on insects in relation to hairy root. Isolations of the hairy-root bacteria were made from white grubs that had been feeding on diseased tissue. Out of 38 isolation trials from the alimentary tracts of white grubs in 1930, only one culture of the bacteria was obtained. The method of isolation consisted in disinfecting the exterior of the insect by immersion for 10 seconds in mercuric chloride, 1 : 1,000, and removing the digestive tract, which was transferred directly to 100 cc of sterile distilled water. The vessel containing the water and digestive tract, after being shaken, was allowed to stand for 30 minutes, when five 1-cc portions were plated on bile agar (*19*). Out of five isolation trials in 1931 from the mouth parts of white grubs which had been eating young hairy-root overgrowth tissue when collected the day before and on which no surface disinfectant was employed, two cultures of the hairy-root bacteria were obtained. Eleven isolation trials from the alimentary tracts of as many white grubs, when no disinfectant was employed, were all negative. These studies are of a limited nature. However, the fact that some insects which habitually feed upon underground plant parts may carry the hairy-root bacteria even for a short time is of significance in the life history of the hairy-root organism.

Isolations from other insects gave negative results. Regardless of the significance that may be attached to these isolation studies, the fact remains that wounds produced are potential infection courts for the bacteria.

Insect repellents—paradichlorobenzene (*9*) and mercuric chloride (*7*, *10*)—were employed in an effort to reduce the amount of insect injury and consequently of hairy-root infection on first-year trees in the nursery. Although 50 and 35 percent reduction in hairy root was secured, the data are omitted because they are insufficient for definite conclusions.

Insect barriers made of treated cloth were tested. Cloth of unbleached muslin, 50 meshes to the inch, was washed to remove the filler, and dried. Strips 4 inches wide were folded and sewed to make a sack approximately 1 inch in diameter and 4 inches long. The sacks were soaked for 30 minutes in a preservative solution of 29 parts (by weight) of copper oleate dissolved in 71 parts of gasoline. This treatment has been successfully used in the preservation of cotton fish nets in both sea and lake water (*38*). The sacks were slipped over the graft unions and fastened at the base by folding and securely wrapping with a strip of adhesive tape 4 inches long by one half of an inch wide. Autoclaved soil was introduced from the top in sufficient amount to keep the cloth from contact with the union. The top of the sack was then folded and wrapped in the same manner as the bottom. Grafts wrapped with string and made from the same supply of scions and roots were used in this experiment. Five hundred grafts were fitted with sacks. An equal number without sacks were planted alongside. The results taken at the close of the season showed about 6 percent of the sacked grafts and 24 percent of the controls infected. The cloth resisted decay until August, when an occasional sack had begun to show disintegration. Along with this treatment there was a growth-retarding action from the use of the sacks of about 4 inches to the tree. Moreover, about 16 percent more of the sacked trees than of the controls died during the summer. Although the reduced amount of disease for the sacked grafts may have been partly due to one or more causes besides the cloth barrier, the evidence appeared to warrant a repetition of this experiment. The following season a similar study gave more reliable results. For the sacked and control trees there were, respectively, 13.9 and 26.0 percent of disease, 75 and 76 percent of stand, and 32.6 and 34.4 inches of average height. With so little difference in stand and height, the difference in percentage of disease appears important. The possibility of antiseptic action by copper oleate upon the hairy-root bacteria was tested but was not found to be significant. These experiments gave added strength to the idea of insect interference.

INFLUENCE OF CONDITION OF HOST

The entrance of the hairy-root bacteria into nursery apple trees was studied in relation to the susceptibility of the host as influenced by (1) the time of the growing season, (2) the age of the tree, (3) the size of the tree, (4) the variety of the tree, and (5) the previous infection of the tree.

The period of the growing season seemed immaterial so far as entrance of the organism was concerned. This was determined by making inoculations at weekly intervals throughout three growing seasons. Each week 10 trees were inoculated in the usual way by introducing the bacteria through scalpel cuts in the stems in five places. The percentage of trees that became infected was used as the measure of the entry of the bacteria. The results for the three seasons are given in table 4. Except at sporadic intervals during the growing seasons studied, the trees permitting entry of the bacteria approached 100 percent, the averages for 1929, 1930, and 1931 being 97, 96, and 98 percent. The apparent exceptions seem to be within the range of experimental error. The rapid falling off in the percentage of disease in September 1929 was probably due to the early dormancy of the trees. Of the inoculations made on September 4 and September 11, 50 and 0 percent, respectively, had become infected by the time of the last observation in November. This idea was verified when in May 1930 it was found that all the trees which had apparently become dormant the September before showed infection. Consequently, 100 percent infection is shown in table 4. The bacteria had entered and overwintered in the wounds, ready to produce infection the following spring. Corresponding inoculations in September 1930 and September 1931 produced disease symptoms in November. It appears, therefore, that bacteria will enter the host during any part of the growing season and will produce infection if the trees have not become dormant.

TABLE 4.—*Wounded apple trees infected by hairy-root bacteria at different periods during 1929, 1930, and 1931*

Date	Trees infected [a]	Date	Trees infected [a]	Date	Trees infected [a]
1929	*Percent*	1930	*Percent*	1931	*Percent*
May 15	90	May 12	100	May 11	100
May 22	100	May 19	100	May 18	100
May 29	100	May 26	100	May 25	100
June 5	100	June 2	100	June 1	100
June 12	100	June 9	100	June 8	90
June 19	90	June 16	50	June 15	100
June 26	100	June 23	100	June 22	100
July 3	100	June 30	100	June 29	90
July 10	100	July 7	100	July 6	100
July 17	90	July 14	100	July 13	100
July 24	100	July 21	100	July 20	100
July 31	100	July 28	100	July 27	100
Aug 7	100	Aug 4	90	Aug 3	100
Aug. 14	...	Aug 11	90	Aug 10	100
Aug. 21	100	Aug 18	100	Aug 17	90
Aug. 28	80	Aug. 25	100	Aug 24	90
Sept. 4	[b] 100	Sept. 1	100	Aug 31	100
Sept 11	[b] 100	Sept 8	100	Sept 7	100
				Sept 14	100

[a] 10 trees were inoculated each week

[b] For explanation see paragraph above table.

The age of the nursery apple tree, in this region where trees are grown only 2 years, did not seem to influence susceptibility to infection by the hairy-root bacteria. Inoculations made in 1929 on first- and second-year trees showed 85 and 93 percent of infection, respectively, and inoculations made in 1930 and 1931 showed 97 percent of infection on both first- and second-year trees. An average of 40 trees, each wounded in five places, was used in each part of the test.

All sizes of growing apple trees were found infected in the same field under natural conditions. Each year, at the end of the growing season, representative first-year trees were selected, examined for disease development, and measured for height. A graphic presentation (fig. 2) of the data accumulated during the 1929 season serves to bring out the distribution of the diseased and healthy trees with respect to the different size groups arbitrarily chosen. For graphing, all the trees were placed in size groups differing from each other by intervals of 4 inches, e.g., (1) less than 12 inches, (2) 12 to 16 inches, (3) 16 to 20 inches, etc. These data showed a fairly even distribution of both diseased and healthy trees over the range of size groups. A similar study on second-year trees (fig. 3) gave similar results. Since all sizes of trees may be found infected in the field it seems logical to conclude that size is not an important factor in susceptibility of trees to infection by the hairy-root organism. Further evidence in support of this conclusion was derived from an experiment in which 311 trees as they came in the row were inoculated; subsequently, 95 percent were found to have become infected. When compared as to size it was found that most of the 5 percent which resisted infection fell into the more numerous middle-sized groups.

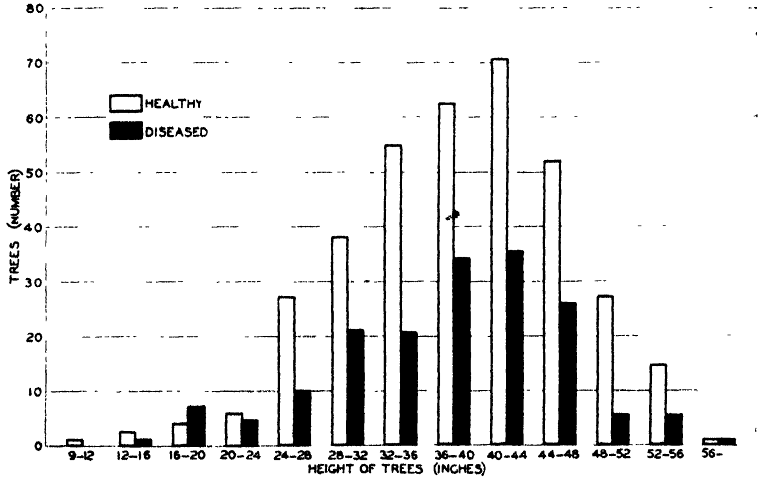

FIGURE 2.—Distribution in 1929 of healthy and hairy-root diseased 1-year-old apple trees in groups based on height of trees in inches.

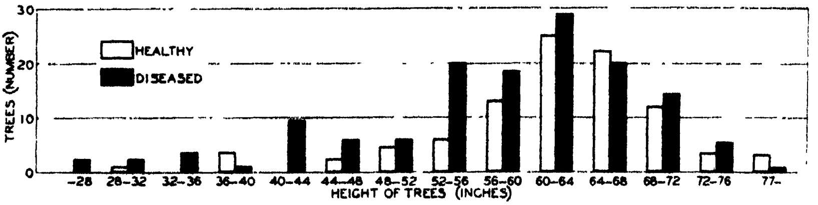

FIGURE 3.—Distribution in 1930 of healthy and hairy-root diseased 2-year-old apple trees in groups based on height of trees in inches.

Varietal susceptibility to infection by the hairy-root organism differed widely among the varieties of trees studied. That not all varieties of apple are equally susceptible to the hairy-root disease has been recognized by different workers, including Hedgcock (*12*), who reported as the result of a country-wide survey that the Ben Davis, Wolf River, and Northern Spy varieties especially were attacked in the nurseries. The present study was made on the relative susceptibility of the varieties of apple trees commonly grown at Topeka, Kans. Late in May 50 inoculations were made in each of 29 varieties in 1930 and in each of 37 varieties in 1931. Twenty-seven of the same varieties appeared in the tests both years. The varietal names were checked with "Standardized Plant Names" (*2*), the local names being retained when the variety did not appear in the standard list. Two names are given for some of the varieties. After 2 months of incubation the overgrowths were cut off even with the stem surface, wrapped in moist paper, and taken to the laboratory. The following points were recorded for each variety: (1) The number of inoculations giving positive reactions, (2) the volume displacement of hairy-root tissue, (3) the wet weight of hairy-root tissue, and (4) the dry weight of hairy-root tissue.

On the basis of the percentage of wounds infected, the different varieties ranged in susceptibility from 12 to 100 percent in 1930 and from 22 to 100 percent in 1931 (table 5). Three of the 27 varieties appearing both seasons (Fameuse, Florence (crab), and Livland Raspberry) gave the same percentage of positive inoculations; three others (Hopa (crab), McIntosh, and Wealthy) gave a lower percentage of positive inoculations the second season; the remaining 20 varieties showed an increase in the percentage of positive inoculations the second season. For example, the Yellow Transparent variety had a susceptibility to infection of 48 percent in 1930, and 82 percent in 1931. On the basis of growth, the varieties showed some difference with respect to one another and considerable difference with respect to the season. The average amount of hairy-root growth for each positive inoculation was calculated on the three bases already given.

Since the three different measures of hairy-root development showed such a close correlation, only the first one need be discussed. The volume growth in cubic centimeters of the different varieties ranged from 0.31 to 1.71 in 1930 and from 1.00 to 4.04 in 1931. Without exception, in the 27 varieties appearing both seasons a considerably larger growth of diseased tissue occurred the second season. When the varieties were grouped the average growth of diseased tissue in 1931 was approximately 300 percent of that of 1930. These studies indicate that varietal differences in susceptibility to the hairy-root organism are important factors in the infection and development of hairy root.

In 1929 50 second-year trees which had become diseased the first season were wounded by deep punctures made with a pointed blade above, below, and at either side of the union, at least 1 cm from the enlargements; 25 other diseased trees were kept unwounded as controls. A group of 25 healthy trees were wounded about the union in the manner described above; 50 others were left unwounded as controls. The soil in which the trees were growing was infested with the hairy-root bacteria. Of the diseased trees, 24 percent of those wounded and 12 percent of those left unwounded showed new infections at the end of the year; of the healthy trees, 28 percent of the wounded and 14 percent of the unwounded became infected. This study revealed the fact that new infections occurred whether the trees were diseased or healthy. Moreover a number of new infections occurred in the same series where no wounds were made, the majority of these infections appearing at the union or above it on the scion. Numerous observations in the field confirm these findings. Apparently previous infection of nursery apple trees does not produce an immunity to later infection. This is in accord with the results obtained by Smith et al. (*36*), Brown (*5*), and Riker (*22*), in their work with crown gall.

TABLE 5.—*Results of tests made during 1930 and 1931 in Kansas on the relative susceptibility of 38 varieties of nursery apple trees to infection by the hairy-root organism* [a]

Variety	Inoculations positive		Average growth of reactions measured by—					
			Displacement		Wet weight		Dry weight	
	1930	1931	1930	1931	1930	1931	1930	1931
	Percent	*Percent*	*Cc*	*Cc*	*Grams*	*Grams*	*Grams*	*Grams*
Anoka	34	76	0.60	1.58	0.51	1.32	0.12	0.27
Arkansas [b]	20	56	.65	1.21	50	1.06	10	18
Baldwin	42	86	31	1.16	.26	1.05	05	21
Beauty (crab)		34		1.30		1.03		19
Ben Davis	72	88	39	1.89	38	1.50	07	27
Cortland	100		.88		80		19	
Delicious	62	94	.45	1.70	36	1.57	07	37
Dolgo (crab)		98		3.51		3.08		66
Early Cooper	98	100	1.71	3.78	1.40	3.43	26	61
Early Harvest	26	68	69	1.56	59	1.33	15	27
Fameuse [c]	94	94	1.49	3.77	1.41	3.46	22	60
Florence (crab)	60	60	1.13	3.33	1.02	3.05	18	51
Gano I [d]	30	100	.50	3.04	45	2.77	07	62
Gano II		92		1.72		1.63		29
Grimes Golden	38	84	.80	1.38	67	1.24	.13	27
Hopa (crab)	38	22	42	1.45	34	1.07	08	20
Hyslop (crab)	98	100	1.39	3.86	1.28	3.72	25	76
Jonathan	56	92	.61	2.59	55	2.39	.10	47
Livland Raspberry [e]	100	100	1.31	2.14	1.19	1.87	21	40
McIntosh	38	36	60	2.12	57	2.08	14	.41
Minkler		70		1.08		1.00		19
Maiden Blush	86	96	89	3.17	78	2.77	15	52
Northwestern Greening		90		2.69		2.35		37
Oldenburg [f]		96		2.73		2.46		41
Rambo	68		36		30		08	
Red Astrachan	88	98	.60	1.53	56	1.23	08	22
Red June		98		2.96		2.58		61
Rome Beauty	20	30	44	1.47	40	95	.07	.23
Stayman Winesap	12	86	.33	2.63	31	2.44	.05	44
Tolman Sweet	54	84	.46	1.88	39	1.71	09	34
Turley		74		1.65		1.28		58
Wealthy	74	72	.51	2.22	.49	1.96	.10	.39
Whitney (crab)	14	68	.57	2.44	.46	2.07	.09	.48
Willowtwig		98		2.61		2.31		.39
Wilson June		96		4.04		3.37		67
Winesap	24	90	42	2.24	.37	2.03	07	44
Winter Banana	60	86	.47	1.00	.40	.87	.08	15
Yellow Transparent	48	82	62	2.02	52	1.83	.11	37
York Imperial	16	66	.44	1.18	39	.94	.10	21
Average [g]	52	78	.69	2.16	.61	1.91	.12	37

[a] 50 inoculations were made on each variety.
[b] Mammoth Black Twig.
[c] Snow.
[d] Black Ben.
[e] Livland Raspberry.
[f] Duchess.
[g] These averages represent the 27 varieties occurring both seasons

LOCATION OF ORGANISM WITHIN HOST

The location of the hairy-root organism in the host tissues was another phase of the life history studied. Studies on the location of the crown-gall organism in the tissues of several hosts were made by Riker (*21*), Robinson and Walkden (*32*), and Ivanoff and Riker (*13*). Because of its similarity to the hairy-root organism, the studies on the crown-gall organism suggested methods of approach for this work.

The hosts selected for this study were Paris daisy and apple. Inoculations were made only on young stems of vigorous plants, from 1 to 4 inches below the apex. The method of inoculation was by needle puncture through a drop of a suspension of the bacteria placed on the surface of the stem. Control punctures were made without the bacteria. Inoculated and uninoculated stem specimens were prepared for study, at intervals of 1 hour, 1, 2, 3, 4, 8, and 16 days after treatment. This material was examined both in the fresh state and when embedded in paraffin after being fixed in formalin acetic-alcohol. Parallel series were prepared in which the inoculum consisted of hairy-root bacteria mixed with *Phytomonas insidiosa* (McCulloch) Bergey et al., a Gram-positive organism, and hairy-root bacteria mixed with india ink. These mixtures, devised to aid in the location of the bacteria in the tissues, are similar to those used by Ivanoff and Riker (*13*). Of a large number of staining combinations tried, including the use of Gram's stain in studying the mixture of organisms, the staining procedure found most satisfactory was to immerse the preparation for about 1 second in dilute safranine, 1:1,000, followed by the light-green counterstain.

Relations of the hairy-root organism with the host are apparently first begun in the liquid released by the wounds. When wounds were made in the stems of Paris daisy and apple, there was a water-soaking of the neighboring tissues similar to that described by Riker (*21*) in tomato. These juices, liberated from the cells by the wound, flooded the neighboring intercellular spaces for a short distance from the wound cavity. The bacteria were observed in the wound cavity and for some distance between the cells in the surrounding host tissues. The bacteria, operating under a complexity of forces, as suggested by Ivanoff and Riker (*13*), become distributed in the wound juices that ordinarily fill the neighboring intercellular spaces.

In the fresh material the bacteria appeared to be located in the intercellular spaces in both Paris daisy and apple stems. For example, 8 days after inoculation a comparison of both Paris daisy and apple tissues which had received control punctures was made. The crushed tissues bordering the wound cavity in both inoculated and uninoculated stems were discolored. The inoculated stems had an intercellular discoloration spreading out into the tissues for a short distance from the path of the needle. It varied from a pale yellow to a brown and was especially marked in the tissues outside the cambium. None of the uninoculated tissues showed this discoloration. From the limited studies made the position of the discoloration, whether in the walls or in the material between the cells or in both, could not be determined with finality. However, in the section examined, it seemed to be present in both places in varying amounts. The extent of the discoloration between the cells from the edge of the wound seemed to correspond rather closely to the area which appeared water-soaked when the wound was made, roughly a distance of 10 to 20 cells. The fact that outside the path of the needle only the inoculated tissue became discolored indicated that this condition was caused by the presence of the bacteria.

The fixed Paris daisy material, whether stained or unstained, was found to contain the bacteria in the spaces between the cells. Sections were cut from 10µ to 20µ in thickness. After inoculation the unstained tissues showed discolorations similar to those of the fresh material. A yellowing of the regions apparently invaded by the bacteria became evident 4 days after inoculation; 8 days after inoculation the color became deeper, almost brown. Staining dyes showed a strong affinity for these regions, so that the bacteria, if present, were obscured. In some sections the use of india ink or Gram-positive bacteria made the invaded intercellular spaces more distinct, although their position and extent seemed to be the same. It was generally found that the discolored regions in 8-day-old preparations took the dyes very readily, making the bacteria difficult to see. Starting from the wound, search was made for the bacteria. Only cells that had been injured were observed to contain bacteria within them (fig. 4, *A*). The bacteria could be traced for short distances into the intercellular spaces. In the pith of Paris daisy there are comparatively large intercellular spaces. Photomicrographs of a cross section and longitudinal section of stems 8 days after inoculation showed deeply stained material in these intercellular spaces (fig. 4, *B* and *C*). With high magnification the bacteria are visible in such locations (fig. 4, *D*).

Studies of the apple stems involved preparations similar to those on Paris daisy, and showed the bacteria to be similarly situated. The extent of migration of the bacteria into the tissues outside the xylem from the path of the wound seemed even more limited than for Paris daisy. Moreover the bacteria were observed to stimulate hyperplasia only in the tissues outside the xylem.

The presence of the organisms between the cells of the tissues bordering the wound stimulated a multiplication of the cells in those localities. This change appeared to begin about 8 days after inoculation. All the tissues were examined less than 16 days after inoculation. Cross sections of stems made 8 to 16 days after inoculation revealed the presence of circular areas of hyperplasia (fig. 4, *E* and *F*) outside the xylem. Variable in number and from one to several cells in radius, these areas were in close proximity to the wound borders and appeared to be about the intercellular spaces containing the bacteria. For a distance of from 1- to 10-cell diameters away from the center of such localities the cells were stimulated to division in a tangential plane. This was the condition 16 days after inoculation. Bacteria have been observed usually at the centers of such areas, which when stained stand out distinctly in strong contrast to the surrounding tissues. The development of hairy root after the formation of the hyperplastic areas is an important phase of the disease requiring further investigation.

In the injured xylem vessels the organisms appeared for a short distance from the wounds in both cross and longitudinal sections of apple and Paris daisy stems. In some longitudinal stem sections of Paris daisy it was common for the bacteria to be present in the vessels to the end of the section. Stem cross sections above and below the wounded area showed the bacteria to be present within the vessels

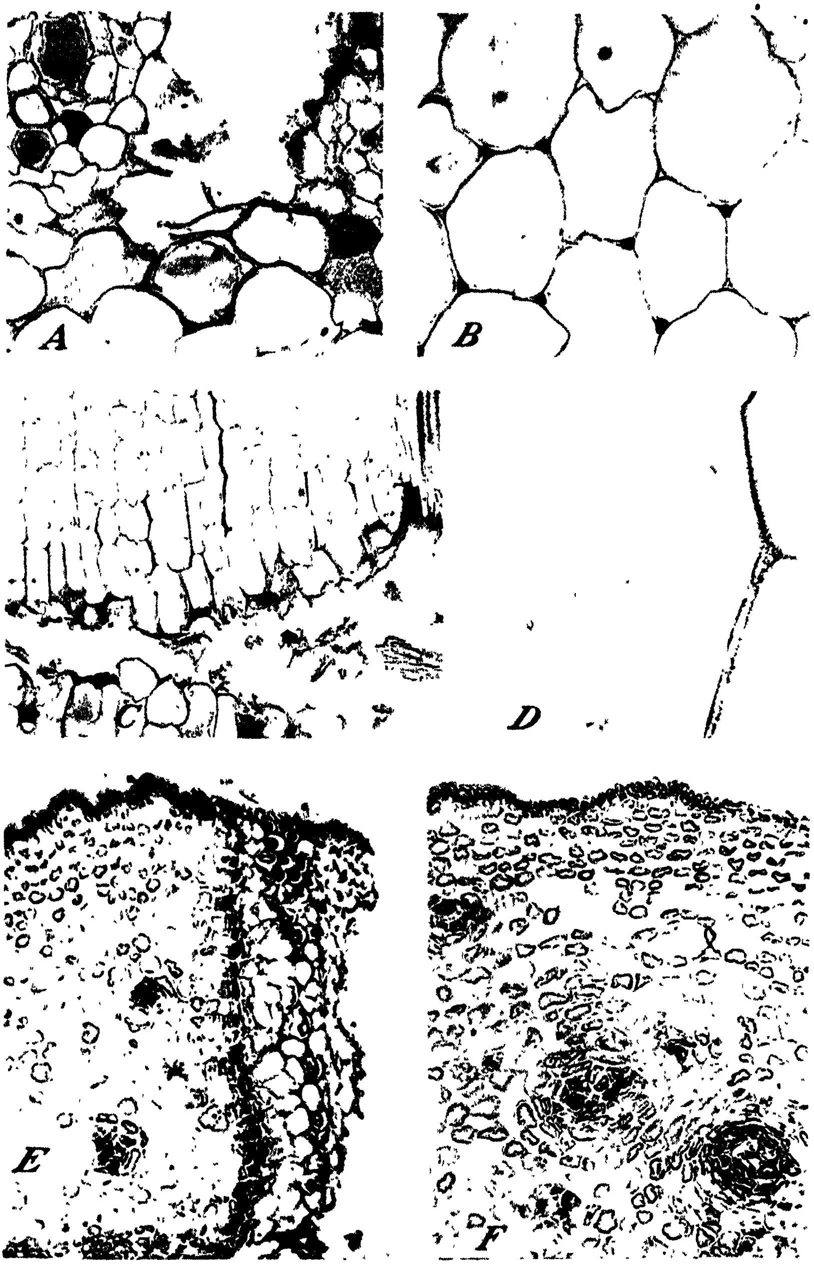

FIGURE 4.—Photomicrographs showing the location of the hairy-root bacteria. *A*, Cross section of a Paris daisy stem 4 days after puncture inoculation, showing the bacteria within injured host cells and in the neighboring vessels. × 270. *B*, Cross section of a Paris daisy stem 8 days after puncture inoculation, showing discolored intercellular spaces which contain the hairy-root bacteria. × 270. *C*, Longitudinal section of a Paris daisy stem 8 days after puncture inoculation, showing discolored intercellular spaces which mark the position of the hairy-root bacteria. × 100. *D*, Longitudinal section of a Paris daisy stem 8 days after puncture inoculation, showing the hairy-root bacteria in the intercellular space. × 550. *E*, Cross section of an apple stem 8 days after puncture inoculation with the hairy-root organism, showing the position of the circular areas of hyperplasia in relation to the host tissues and to the wound. × 200. *F*, Another cross section of an apple stem showing the position of the circular areas of hyperplasia in the host tissues above and below the wounds. × 200.

(fig. 4, A). Because no changes in these tissues were observed, the presence of the bacteria in the vessels appeared to be of no importance in bringing about infection.

Several thousand inoculations made on unwounded apple trees failed to produce infection. Symptoms of hairy-root infections induced by inoculation have invariably originated at the point of inoculation. This characteristic of hairy root is in accord with the limited migration of the bacteria from the wound cavity found in the studies on the location of the bacteria.

A study of the location of the bacteria in the older tissue was continued with bacteriological technic.

The bacteria seemed to be most abundant in the soft outer tissues of the enlargement. This was determined from isolation studies in which the writer used the technic described earlier. The interior of the basal enlargements of hairy root consisted chiefly of xylem elements. For some distance beneath the surface two tissues are paramount in the enlargement—the hard, white, woody interior, and a soft, almost colorless, cortical exterior of varying thickness. Exposure to the air for a few minutes caused these soft outer tissues to become brown as if oxidized. The vascular tissues remained unchanged (fig. 5).

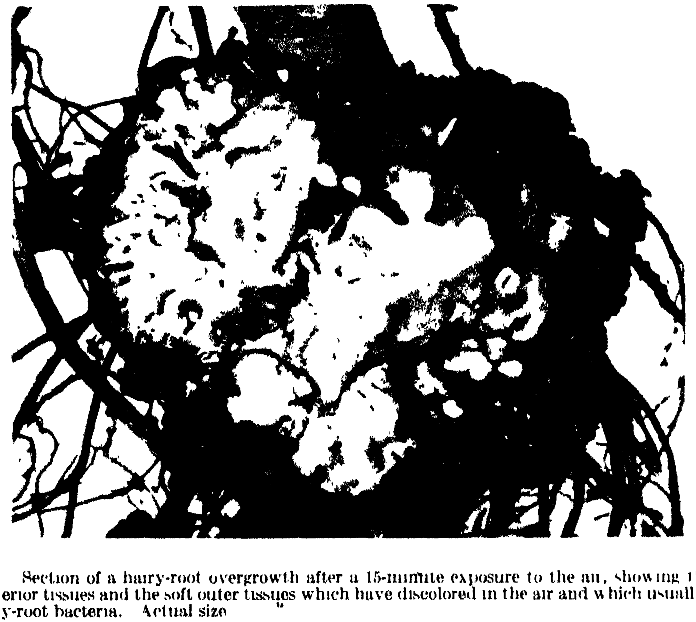

FIGURE 5. Section of a hairy-root overgrowth after a 15-minute exposure to the air, showing the white, hard interior tissues and the soft outer tissues which have discolored in the air and which usually contain the hairy-root bacteria. Actual size

The bacteria were isolated consistently from the surface and subsurface parenchymatous tissues of the enlargements. In order to clarify the position of the bacteria in the enlargements, isolations were made from different tissues. Different kinds of enlargements common to the nursery, approximately 50 percent of which were hairy root and the remainder principally wound overgrowth, were taken at random and isolations made. In a series of 152 isolation trials, 131 series of platings were made from the surface, 152 from the subsurface, and 152 from the deep interior. The surface tissue yielded the largest percentage of the organism, or 39 percent (table 6). In sampling the surface tissue soil particles were sometimes unavoidably included. The subsurface parenchymatous tissue was removed aseptically and yielded infectious bacteria in 38 percent of the isolation trials. From the deep vascular tissue the bacteria were obtained only from discolored areas where soil particles were imprisoned. It should be noted in the table that nonpathogenic bacteria, *Bacillus radiobacter* Beij. and Van Deld., were uniformly obtained in considerable quantity. The significance of these nonpathogenic bacteria has not been determined. However, the location of the bacteria at the surface of the enlargements is of importance in relation to the soil.

TABLE 6.—*Summary of isolation studies from the different tissues of representative enlargements found in the nursery* [a]

Tissue examined	Isolation trials	Enlargements yielding—		
		Phytomonas rhizogenes	Nonpathogenic bacteria	No bacteria
	Number	*Percent*	*Percent*	*Percent*
Surface parenchymatous	131	39	32	29
Subsurface parenchymatous	152	38	28	34
Deep interior vascular	152	[b] 1	[c] 2	97

[a] No crown galls were included among these specimens.

[b] The deep interior tissue yielded *Phytomonas rhizogenes* only from discolored tissue containing soil particles.

[c] Nonpathogenic bacteria also were obtained only from discolored tissue containing soil particles.

SOIL RELATIONS OF ORGANISM

The bacteria were found to be abundant on the surface of the hairy-root enlargements. The most promising method of studying this problem seemed to be dependent upon the production of infections under approximately aseptic conditions away from common soil contaminants. The production of hairy root under these conditions has been worked out by Riker, Hildebrand, and Ivanoff (*29*), where the technic is given. Examples of the diseased specimens produced by this method are described. Briefly, the technic consisted of fitting glass cylinders around the stems of apple and sealing them so as to exclude all micro-organisms, except the hairy-root bacteria, used in making the inoculations. At intervals of 9, 10, 13, 15, 17, 19, and 21 weeks after inoculation the specimens were taken for study. The surfaces were sterilized by immersion for 15 minutes in a 20-percent solution of a sodium hypochlorite preparation, known as "Bacillikill", which contains 3.5 percent of sodium hypochlorite by weight. The disinfectant was removed by washing through three changes of sterile distilled water. In an effort to recover the organism, bile-agar platings were made of the sterile distilled water in which the specimens were allowed to be immersed for intervals of 10 minutes, 1 hour, 4 hours, 1 day, and 2 days.[5] Hairy-root bacteria were obtained in the washings of all the specimens and for all the washing intervals tested. One control specimen gave off no bacteria; a crown-gall specimen yielded the crown-gall bacteria.

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[5] This technic was developed after consultation with W. M. Banfield, Department of Botany, University of Chicago.

A parallel exit study, which was conducted on disease specimens produced in the soil, gave similar results. Four specimens, inoculated, respectively, 2, 4, 6, and 8 weeks previously, were used in this study. These specimens were washed free from soil particles in such a manner as to produce no injuries. They were then sterilized and washed free from the sterilizing solution as in the preceding experiment. The specimens were placed for 1 hour in sterile distilled water before the water was plated. After an incubation period of 1 week the hairy-root bacteria were obtained from the platings of specimens of all four ages. The proof of the identity of the bacteria saved from these isolations was established by positive inoculations on apple.

The bacteria were found rather often in the soil near the hairy-root formations. In 1929, 10 isolation trials were made at each of three 10-day intervals, starting May 28. The technic employed was as follows: For each trial a sample of about 500 g of soil was removed from around a hairy-root formation and taken to the laboratory. After a thorough mixing, a sample of 20 g was weighed into a flask containing 200 g of sterile distilled water. This was shaken and allowed to settle for about 1 hour. One cubic centimeter of the supernatant liquid was then transferred to each of three Petri dishes. Loop dilutions from each of these were made to three other dishes, respectively, containing 1 cc of sterile distilled water. Bile agar was added, and the plates were then allowed to incubate at room temperature for 1 week, after which an examination was made. The percentage yields of the hairy-root organism in the three successive trials of this experiment were 40, 80, and 60, respectively. The bacteria were obtained in 18 of the 30 attempts, or 60 percent.

A similar study, consisting of 10 trials conducted on soil taken from around healthy unions, were all negative. Since the bacteria were obtained relatively easily from random samples from the nursery soil this may seem unusual. However, in securing these samples soil was taken from around trees of a poorly knotting variety where there was less chance of soil contamination.

A 3-day-old growth of bacteria from six large prescription bottles was poured into a cubic foot of soil stored in the field, and the same treatment was given a similar amount of soil kept in a storage cellar These soils were inoculated in October 1930, and three isolations were subsequently made from each soil at monthly intervals, through April 1931. The soil stored in the cellar yielded the bacteria in 20 out of 21 trials; the field soil yielded the bacteria in 19 out of 21 trials. It is apparent from these experiments that the bacteria may overwinter in soil kept in the field or with nursery stock stored in the cellar.

The longevity of the bacteria in field soil has been found to exceed 1 year both in Wisconsin and in Kansas. At Madison, Wis., steamed soil inoculated with the hairy-root bacteria in the summer of 1928 yielded the bacteria in 2 out of 4 trials in April 1929 and in 1 out of 4 trials in October 1929. Field soil inoculated in the summer of 1929 yielded the bacteria in 1 out of 3 trials in October 1930, and in 0 out of 3 trials in April 1931. In 1930 an attempt was made in Kansas to recover the hairy-root organism from the soil of four fields that had grown or were growing nursery apple trees. Fields 1, 2, 3, and 4 had grown trees during 1927 and 1928, 1928 and 1929, 1929 and 1930, 1930 and 1931, respectively. At five different intervals (June 3, June 17, July 11, July 30, and August 28), 10 soil samples from each field were collected at random from a depth of about 4 inches and then mixed for each field. Platings were made according to the technic of the previous season, except that four instead of three 1-cc samples were taken for each flask. The results show that the bacteria were present but were not very abundant in three of the fields. Briefly, from a total of 20 trials for each field, the hairy-root bacteria were obtained in 0, 20, 25, and 30 percent, respectively. Only in field 1, from which the trees had been removed 2 years previous, were no bacteria obtained. The presence of the bacteria in field soil from which the trees had been removed a year previous (field 2) and their absence in soil which had grown trees 2 years previous (field 1) indicated the length of time after inoculation that bacteria could be isolated from the soil. Isolations were again attempted in May 1931 from fields 2, 3, and 4 and from the soil of the new graft field, which the season before had grown apple seedlings. Field 2 failed to yield the bacteria, although it had done so the previous season. Fields 3 and 4 and the new graft field yielded the bacteria in 25, 25, and 50 percent of the trials, respectively. The presence of the bacteria in the graft field in May in so short a time after the grafts were planted may perhaps be traced to the growth of seedlings in this field the previous season. These results correspond in general to those secured by Patel (*20*) and Banfield (*3*) for the length of time crown-gall bacteria could survive in unsterilized soil.

DISTRIBUTION AND TRANSMISSION OF ORGANISM

Although nursery stock may be inspected and diseased trees destroyed, it is difficult to find the incipient stages and impossible to find recent infections. Moreover, healthy plants may carry the bacteria on their roots. In 1929 a consignment of apparently healthy Wealthy apple grafts were planted at Madison, Wis. (*23*), in beds of soil that had been steamed. At the close of the growing season some of the trees had overgrowths at their unions. Twenty trees showing these malformations were selected at random. Isolations from 14 yielded typical hairy-root bacteria. The probability that these bacteria entered the trees from the steamed soil is remote, hence they were probably carried either in the unions or on the surface of the roots. It seems fairly obvious that the part played by shipments of nursery stock is important in the widespread dissemination of the disease. This may account for its presence in practically every region where the host plant is grown.

The seedling root was found to be one medium of transmission of the causal organism from one crop to the next. Siegler and Piper (*35*) reported that "the seedlings may in nature carry surface-borne organisms in quantities sufficient to be considered an important source of inoculum." Isolation trials from washings from certain seedling roots have yielded the bacteria. Three-inch pieces from 26 different seedling roots were placed in test tubes partly filled with sterile distilled water. Platings were made from the water 4 hours later. Hairy-root bacteria were obtained from 24, or 92 percent, of the specimens, indicating that the seedling is at least one of the primary sources of inoculum. This and other evidence obtained from these life-history studies points to grafting time as the time when the principal primary infection of the apple tree takes place. The presence in the soil of primary infections, resulting from the entrance of the bacteria at grafting time, together with the various wound-producing agencies, are the factors which would seem to account for the secondary spread of the disease.

SUMMARY

In studies of infectious hairy root special consideration has been given to the life history of the causal organism in relation to its pathogenesis on nursery apple trees.

The differentiation of hairy root from crown gall and from wound overgrowth has been repeated and confirmed.

The entrance of the bacteria into the host plants was found to be accomplished only through wounds. The bacteria were able to produce infection if placed only on the surface of the injuries.

The type of wound apparently made no difference in the kind of overgrowths, but did influence somewhat (1) the length of the incubation period, (2) the percentage of inoculations producing infection, and (3) the size of the reaction. Extremely shallow wounds were found to be poor infection courts.

Wounds on the underground stems of nursery apple trees in the field remained open for infection a relatively short time, averaging about 2 days.

Callus was found ordinarily to be a barrier against the invasion of the hairy-root organism. Shallow wounding of callus only occasionally resulted in infection by the bacteria.

Insects appeared important in producing injuries that led to hairy-root infection. Root-feeding insects have been commonly observed during three growing seasons in the soil about nursery apple trees. During the study of diseased and healthy trees, insects frequently have been seen eating hairy-root and healthy tissue. Infected lateral roots were occasionally observed in positions that could have been reached only by insects. The development of new infections throughout both the first and second growing seasons in Kansas was most easily explained as due to the agency of insects.

Several experiments suggested by the observations on insects indicated a relation of insects to hairy root. The hairy-root bacteria were isolated from white grubs. Isolations from other insects gave negative results. Insect repellents in preliminary trials reduced the amount of hairy-root infection. Insect barriers considerably reduced the amount of hairy root.

The time of the growing season seemed immaterial so far as the entrance of the organism was concerned.

The age of the nursery apple trees in Kansas, where trees are grown only 2 years, did not seem to influence susceptibility to infection by the hairy-root organism.

All sizes of nursery apple trees were found infected in the same field under natural conditions and all sizes became infected when inoculated with the causal organism.

Varietal susceptibility to infection by the hairy-root organism differed widely, ranging from 12 to 100 percent for the 29 varieties studied in 1930, and from 22 to 100 percent for the 37 varieties studied in 1931.

Previous infection of nursery apple trees apparently did not prevent subsequent infection.

The hairy-root organism appeared to begin its relations with the host tissues in the liquid released by the wounds.

The bacteria appeared to be located in the intercellular spaces in both Paris daisy and apple stems.

The presence of the organisms between the cells of the tissues bordering the wounds stimulated a multiplication of the cells in those localities, which resulted in the formation of somewhat circular areas of hyperplasia.

The development of infections without wounds has not been observed on apple trees.

The bacteria were isolated from the surface and subsurface parenchymatous tissues of the enlargements.

The bacteria were found to be abundant on the surface of the hairy-root enlargements.

The bacteria were found often in the soil near the hairy-root formations.

The bacteria overwintered in soil kept either in the field or with nursery stock in the storage cellar. The longevity of the bacteria in field soil which had been steamed or left untreated has been found to exceed 1 year.

The bacteria may be spread long distances by shipments of nursery stock The seedling root was found to be one medium of transmission of the causal organism from one crop to the next.

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SEASONAL DEVELOPMENT OF HAIRY ROOT, CROWN GALL, AND WOUND OVERGROWTH ON APPLE TREES IN THE NURSERY[1]

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INTRODUCTION

The seasonal development of hairy root, caused by *Phytomonas rhizogenes* Riker et al., crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. (synonym, *Bacterium tumefaciens* Smith and Town.), and wound overgrowths on apple trees in the nursery has been studied in relation to certain factors that might influence the initiation, development, and control of these diseases. This work was undertaken as a part of the program on the complex graft-knot problem. After the differentiation of these various diseases from one another, a successful effort was made by the senior writer and his associates (*8*, *10*)[3] to induce them at will under controlled conditions. A study was then made by Hildebrand (*4*) of the life history of the hairy-root organism in relation to pathogenesis, with special emphasis on the mechanism of infection. In connection with these lines of work, it appeared desirable to know at what times these overgrowths on nursery apple trees were initiated, under what conditions they developed, and the relative importance of the infections periods.

This study was made in eastern Kansas near the center of the region where piece-root apple grafts are planted in large numbers, where apple seedlings are grown, and where hairy root has occurred with a frequency satisfactory for detailed study. Control measures which had proved adequate in other places often either failed or were only moderately successful in this locality. This fact emphasized the value of the location for studying one of the most difficult phases of control; consequently the analyses presented are of a severe rather than an average situation. The observations and experiments carried out in considerable detail in eastern Kansas were correlated with findings in other nurseries. At least once every fall the results were compared with those secured in Iowa, Minnesota, Missouri, Nebraska, Kansas, Oklahoma, and Wisconsin.

A preliminary statement of some of the earlier phases of this work has already appeared (*11*).

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[2] The writers are indebted to Dr Sarah L. Doubt, Department of Botany, Washburn College, for laboratory facilities, and to Eugene H Herrling, Department of Plant Pathology, University of Wisconsin, for preparing the illustrations

[3] Reference is made by number (italic) to Literature Cited, p 912

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EXPERIMENTAL PROCEDURE

The procedure followed in these studies is an adaptation of that described by Keitt and Jones (*5*). The methods used were selected in consultation with Dr. Keitt.

ENVIRONMENT

The histories of the fields employed varied somewhat. The field in which experimental grafts were planted in 1929 had carried crops of potatoes in 1926, 1927, and 1928. The field in which experimental grafts were planted in 1930 had previously grown three successive crops of corn. The field which was used for experimental grafts in 1931 had grown corn in 1928 and 1929 and apple seedlings in 1930.

The soil reaction was tested from time to time in the three fields in which experimental grafts were planted. The reaction was approximately pH 5.0 in all the fields. The different tests showed comparatively little variation.

Air temperatures were recorded by a thermograph housed 4 feet above the ground in an instrument shelter located among the first-year experimental trees.

The relative humidity of the air was recorded by a hygrograph corrected by the use of a sling psychrometer (U.S. Weather Bureau pattern) and Marvin's (*6*) psychrometric tables.

Soil temperature at the level of the graft union, between 4 and 5 inches beneath the soil surface, was recorded with a soil thermograph. All the instruments were checked daily.

Soil-moisture samples were taken daily from about 4 inches below the surface. The water in the soil was measured in terms of the moisture-holding capacity. With a technic like that used by Riker (*7*), the moisture-holding capacity of the soil in each of the three experimental fields was found to be approximately 38 percent of the oven-dry weight. The amount of water in the daily samples was measured by Bouyoucos' (*3*) rapid method and calculated in terms of the moisture-holding capacity.

The rainfall for each 24-hour period was measured with a rain gage having a 3-inch cup.

HOST DEVELOPMENT

The plant used for all of these studies was the Yellow Transparent variety of apple. Since the diseases studied occur most frequently at the union, the experimental trees were all grown from piece-root grafts made with Kansas seedlings.

The factors of host development considered were the height of the tree and the diameter near the ground level. Measurements were taken at 2-week intervals on 300 first-season and 300 second-season trees. The same trees were used for successive measurements. The height in inches was measured from the original scion where growth began to the top of the main stem. After the early stages of development had passed, the lower point corresponded rather closely with the soil level and was chosen to avoid variations in measurements due to changes in the soil level incident to cultivation. The diameters of the trees in sixteenths of an inch were measured with a nurseryman's caliper about 1 inch above the point from which the new growth left the original scion. This location on the stem was chosen because it was easy to find, was usually just above the ground level, and was generally the place where the stem had the greatest diameter.

STIMULATION OF OVERGROWTHS

Nonparasitic overgrowths were induced in two ways. In some cases aluminum-alloy wire was wrapped twice about the scion a short distance above the union. In other cases a cut about half-way through the scion was made with an upward pull of the knife so that a wedgelike projection of tissue extended down. Direct healing was prevented by inserting a strip of adhesive tape in the cut. This cut produced a result similar to that often found in a poorly fitted graft.

The parasitic overgrowths were induced by inoculations with pure cultures of the causal bacteria. Crown gall was induced with culture A-1 of *Phytomonas tumefaciens*, which was grown from an individual cell. Hairy root was induced with culture C-1 of *P. rhizogenes*. This culture was also grown from a single cell. An account of the purification of these cultures and of their bacteriological characters has already appeared (*14*).

Inoculations at grafting time were made by smearing the cut surfaces of the scions and roots with a suspension of bacteria before these parts of the graft were fitted together and the union was wrapped.

The experimental blocks of trees consisted of a series of parallel rows, each row of which was used for the study of a certain factor, as explained later. Thus on a given day 10 trees in the first row were inoculated with the crown-gall organism, 10 trees in the second row were inoculated with the hairy-root organism, 10 trees in the third row were inoculated with a mixture of the hairy-root and crown-gall organisms, 10 trees in the fourth row received control wounds, 10 trees in the fifth row were girdled with wire, and 10 trees in the sixth row were cut and taped to stimulate wound overgrowth. On successive weeks during the season similar treatments for the production of the overgrowths were made on adjacent trees under comparable conditions.

The method of inoculation during the growing season was as follows. A trench to the depth of the union was made in the soil about 3 inches away from the trees on one side of the nursery row. The trench was opened this distance away to prevent injury to the main stems of the trees while digging. The soil was removed from around the individual trees with a dibble and by hand. Any particles that remained on the main stem were wiped away. Inoculum from a culture of the bacteria was applied with a cotton swab to the stem surface, usually in five places spaced about 1 inch apart. With a scalpel held at an angle, two thrusts were made through each of the drops of culture deep into the stem. Control wounds were made in a similar manner except that no bacteria were employed. During the 1930 and 1931 seasons strips of adhesive tape were applied over both the inoculated and the control wounds to reduce chance contamination from the soil and to keep out insects. Promptly after inoculation the soil was thrown back into the trench so as to cover the topmost wound by about 2 inches.

DEVELOPMENT OF ARTIFICIALLY INDUCED OVERGROWTHS

After the inoculations attention was given to the incubation periods necessary for the development of distinct symptoms.

The incubation period for crown gall was determined by making inoculations at weekly intervals throughout the season with a single-cell culture, A-1, of the crown-gall organism. In 1929 the first inoculations were made, as described earlier, on May 15 and the last on September 11. The corresponding first and last inoculation dates for 1930 were May 12 and September 8, respectively, and for 1931, May 11 and September 14. Because of the similarity in appearance of callus, crown gall, and hairy root, especially in the early stages, the inoculations were not considered positive until the crown galls had a radial extension of 4 mm. This is in accord with the criteria used by Riker, Hildebrand, and Ivanoff (*10*). The incubation time given in the charts (figs. 1, 2, and 3) for any one period is the average time required for the positive reactions from 50 inoculations. Minimum and maximum times are recorded in figure 4. Approximately 55 percent of the inoculations gave positive reactions.

The development of crown gall after inoculation was noted at weekly intervals for the first 6 weeks, during the early disease stages, and at biweekly intervals thereafter. The number, character, and radial extension of the overgrowths were recorded at successive intervals during the season.

The incubation period of hairy root was determined after making inoculations with a culture (C-1) grown from a single cell of the hairy-root organism. The methods and intervals of inoculation were the same as for crown gall. The incubation period for hairy root was recorded in two ways: (1) The time required for a radial extension of 3 mm and for the appearance of root primordia is indicated on the charts by a small circle in the line indicating the incubation period, and (2) the time required for the development of roots 1 cm long is shown at the end of the incubation period. The incubation time given for any one period is the average time required for the positive reactions from 50 inoculations. The minimum and maximum times are recorded in figure 4. Approximately 70 percent of the inoculations gave positive results.

Similar studies were made of the incubation periods and development of the reactions when mixtures of crown-gall and hairy-root bacteria were used for the inoculum.

The host reactions to uninoculated control wounds made in parallel series were examined as controls on the hairy-root and crown-gall series. The procedure was the same except that no bacteria were employed.

The host reactions to wire girdles and to knife cuts with tape inserts were followed in parallel series and at similar time intervals.

The development of disease from inoculations with the hairy-root bacteria at grafting time of 300 string-wrapped and 300 tape-wrapped grafts was observed at monthly intervals during 1930 and 1931.

NATURAL OCCURRENCE OF OVERGROWTHS

The natural occurrence of hairy root was determined in 1929 through biweekly examinations of the unions of 300 trees grown from string-wrapped grafts. In 1930 and 1931 monthly examinations were made of the unions on 1,200 trees, including 300 first-year and 300 second-year trees grown from string-wrapped grafts, and 300 first-year and 300 second-year trees grown from tape-wrapped grafts.

Observations were made at stated intervals by removing the soil from about the union in the manner previously explained. If a tree was accidentally injured in the process, this fact was noted and data were taken accordingly. At the end of the experiments comparisons were made between the percentages of overgrowths on trees examined at various intervals and on those left undisturbed. The variations ranged from 0 to 10 percent. Consequently, it appears that injuries incident to the periodic examinations introduced little if any error into the results secured.

INSECT RELATIONS

Insect surveys were made of the soil in which the experimental trees were growing. In 1929 the work was begun intensively on May 5 and carried on until July 12, when it was discontinued. Subsequently only insects that appeared in connection with the other work were collected and preserved. However, the survey was carried through the season in 1930 and 1931. At biweekly intervals three areas of soil 9 inches square and 6 inches deep were taken at random from around three first-year trees. These samples were mixed and one third of the mixture was taken to the laboratory for examination. All insects observed while the larger sample was being divided were also taken. The soil was minutely gone over by spreading it on light brown paper. The insects present were collected and placed in vials containing 70-percent alcohol, for later identification.[4] The observations in 1930 were begun May 29 and finished September 18. The first examination in 1931 was on May 11 and the last on September 29.

EXPERIMENTAL RESULTS

The more significant aspects of the results obtained are considered under the following heads: (1) Seasonal variations, (2) incubation periods, (3) appearance of overgrowths at different stages of development, and (4) date of natural infection. The records are given in figures 1 to 5, inclusive.

SEASONAL VARIATIONS

Seasonal variations were large during the 3 years covered by these studies. These variations provided opportunities for observing the effect of seasonal changes both at different times in the same season and at corresponding dates in different seasons (figs. 1, 2, and 3). Since the details are recorded in the figures, only the grosser aspects are discussed here.

The air temperatures during 1929 at Topeka, Kans., were generally below those of the average year in that locality. On the other hand, the air temperatures during the season of 1930 were generally above normal, favorable growing temperatures extending into late October. Although the season of 1931 was considerably warmer in June than the previous two seasons, it was about normal in July, below normal in August, and considerably above normal in September.

The average air-humidity readings for the season of 1929 were much higher than those of the two subsequent seasons. During 1930 the average air humidity was the lowest of the three seasons. But for the month of June the 1931 season had an average air humidity falling between those of the preceding two seasons.

The soil temperatures at the level of the graft unions quite naturally followed the trends of the air temperatures. During the day the soil temperatures lagged behind the air temperatures, but at night the opposite relation occurred. As was expected, the extremes of soil temperature were much closer together than those of air temperature.

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[4] The identifications were made by C. L. Fluke, Jr., and E. M. Searls, of the University of Wisconsin.

Soil-moisture data were closely correlated with those of soil temperature and rainfall. During 1929, because of the relatively low soil temperature and high average rainfall, the soil-moisture values were generally the highest of the three seasons. In 1930, because of relatively high soil temperature and low average rainfall, the average soil humidity was the lowest. High soil temperature in June 1931 was accompanied by low soil moisture, largely, perhaps, because of the extremely low rainfall during that month. In September, because of relatively high soil temperature and relatively heavy rainfall, the soil moisture ran a middle course.

Rainfall was rather variable during the three seasons. It was above normal in 1929 and below normal in 1930. In 1931 it was below normal in May and June and approximately normal the remainder of the season.

The host development of nursery apple trees for the 1929 season, according to the local nurserymen, was the largest of any season over a period of 40 years, despite the fact that the trees went into dormancy earlier than in the two following seasons. The large growth was accomplished under conditions of higher soil moisture and somewhat lower temperature than usually obtained. There was no slack period due to soil moisture or temperature conditions. The growth of the trees in 1930 suffered because of the drought and abnormally high temperatures in July and August. However, considerable growth was made during September and October, which partially offset the less favorable period. In 1931 the less favorable period for host growth was in June, when abnormally high temperatures and low soil moistures prevailed. Because of a well-distributed rainfall throughout the remainder of the season the trees overcame much of the handicap of July and August and approached the large growth of the 1929 season. The high temperatures and normal moisture conditions of late fall enabled the first-year trees to make more than average growth, and the second-year trees to make the largest growth of the three seasons. The height and caliper measurements were closely correlated during the three seasons, with one exception. The early fall of 1929 caused the trees to stop growth in height in late September, but the growth in diameter continued into October. Records of the growth of the trees are given in figures 1, 2, and 3.

Insect surveys showed that a number of different kinds of root-chewing insects were present during the growing seasons of 1929, 1930, and 1931. Since the larvae only were found in most instances, it was not possible always to determine the species or even the genus. According to information received from C. L. Fluke, Jr., and E. M. Searls, of the University of Wisconsin, the insects considered most important from the standpoint of opening infection courts were the white grubs (Phyllophaga), wireworms (Elateridae), and fungus gnats (Mycetophilidae). White grubs and wireworms were continually present, but were more abundant in June, early July, and September than in May and August. Fungus gnats were more commonly associated with crown gall and were less generally present than the white grubs or wireworms. From these studies it appears that various insects capable of opening infection courts for crown-gall or hairy-root bacteria were present in varying abundance throughout the growing seasons. The relation of insects to crown-gall infection on raspberries has been discussed by Banfield (*1*, *2*). A similar relation to hairy-root infection on apple trees has been investigated by Hildebrand (*4*).

INCUBATION PERIODS

The time required for the development of excess callus and wound overgrowth following wire girdling or cuts was 2 weeks or more, depending upon the rapidity of the growth of the plant.

Crown-Gall Organism

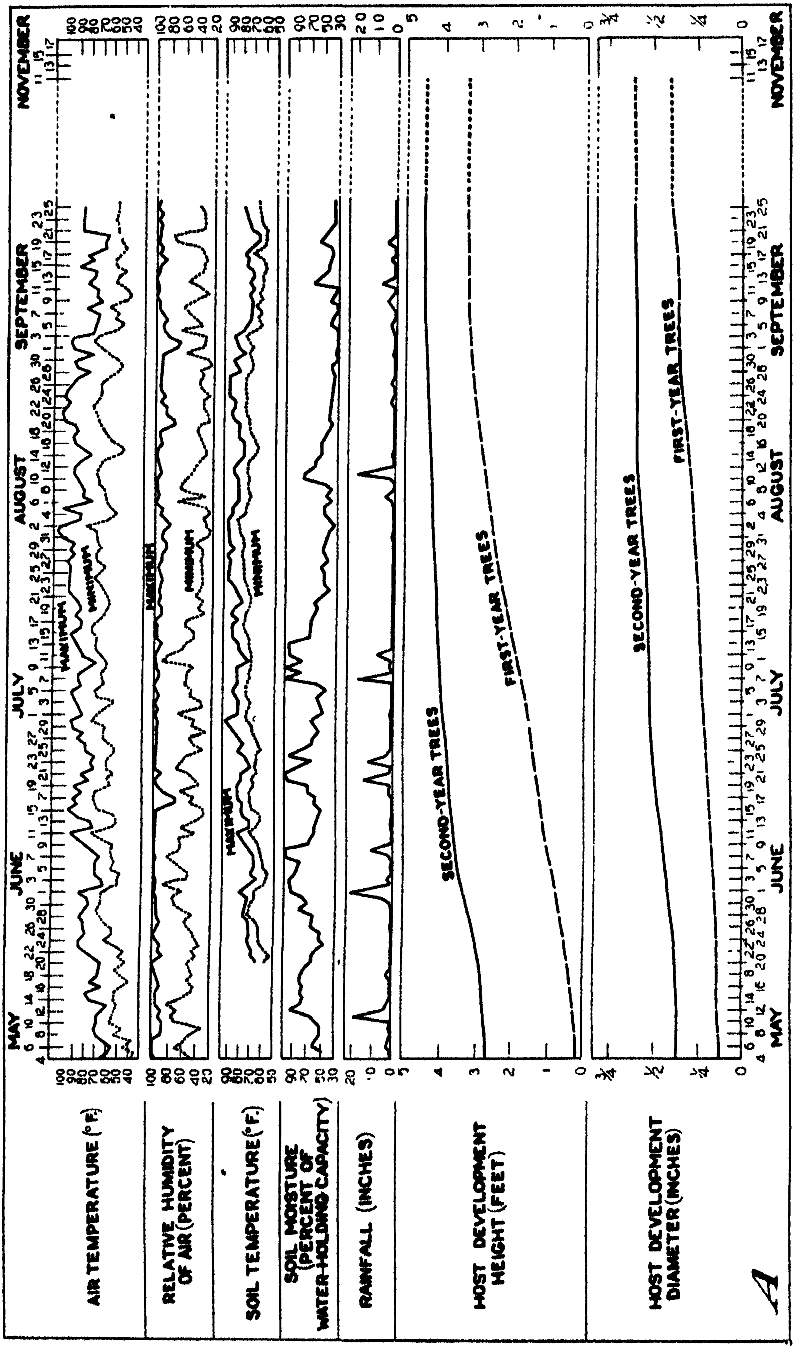
The incubation periods of the crown-gall organism were on an average much longer in the earlier and later parts of the season than in the middle. An inoculation was considered positive only when the gall had a radial extension of 4 mm after 3 weeks (fig. 8, *F*). The minimum and maximum periods are given in figure 4. Doubtless larger numbers would have produced more regular curves. Charts giving the average incubation periods (figs. 1, 2, and 3) show what appears to be a rather close correlation between short incubation periods and warm weather. Only slight differences were noticed in the length of the incubation periods for corresponding weeks in the different growing seasons.

These incubation periods are concerned only with vigorously growing trees. Inoculations on trees which were making little or no growth did not induce disease until the trees began to develop. Inoculations on such trees were often negative, but reactions have sometimes appeared after normal incubation from the time growth began. Thus after inoculations made late in the season overgrowths might not appear until the following spring. The incubation periods on trees growing under favorable conditions had a definite maximum period after which no infection appeared and which seemed to be correlated with the rapidity in growth of the tree and with temperature.

Hairy-Root Organism

The incubation periods for the hairy-root organism were followed in the same way as for the crown-gall organism. The averages are shown in figures 1, 2, and 3, and the minimum and maximum periods in figure 4. The average length of time necessary for development of roots 1 cm long ranged from approximately 3 weeks in the warm period of the growing season to 9 or more weeks in the cool periods at the beginning and end of the season. Two stages in the incubation period of hairy root were observed. The first stage, indicated by a circle on the charts (figs. 1 to 3), was reached when the average hairy-root enlargement had a lateral extension of approximately 3 mm and when root primordia were beginning to show (fig. 6, *A*, after 3 weeks). The second stage in the incubation period was reached when the hairy roots had a lateral extension of 1 cm (fig. 6, *A*, after 5 weeks). As in the case of crown gall, only slight differences were noticed in the length of the corresponding hairy-root incubation periods in the different seasons.

Experiments were conducted to determine when disease would develop from inoculations made at the time of grafting. These trials were made in 1930 and 1931 on both string-wrapped and tape-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RAINFALL (INCHES)
HOST DEVELOPMENT HEIGHT (FEET)
HOST DEVELOPMENT DIAMETER (INCHES)
MAXIMUM
MINIMUM
SECOND-YEAR TREES
FIRST-YEAR TREES
MAY
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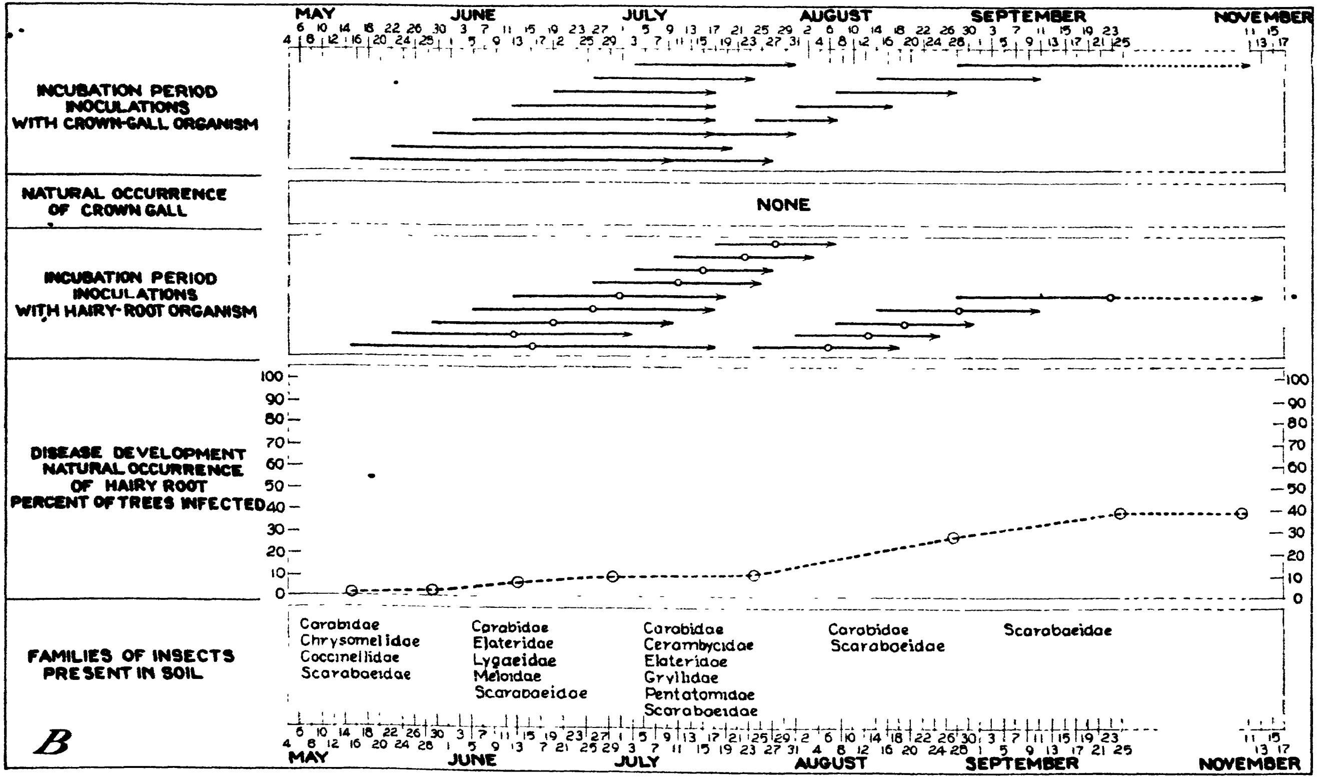
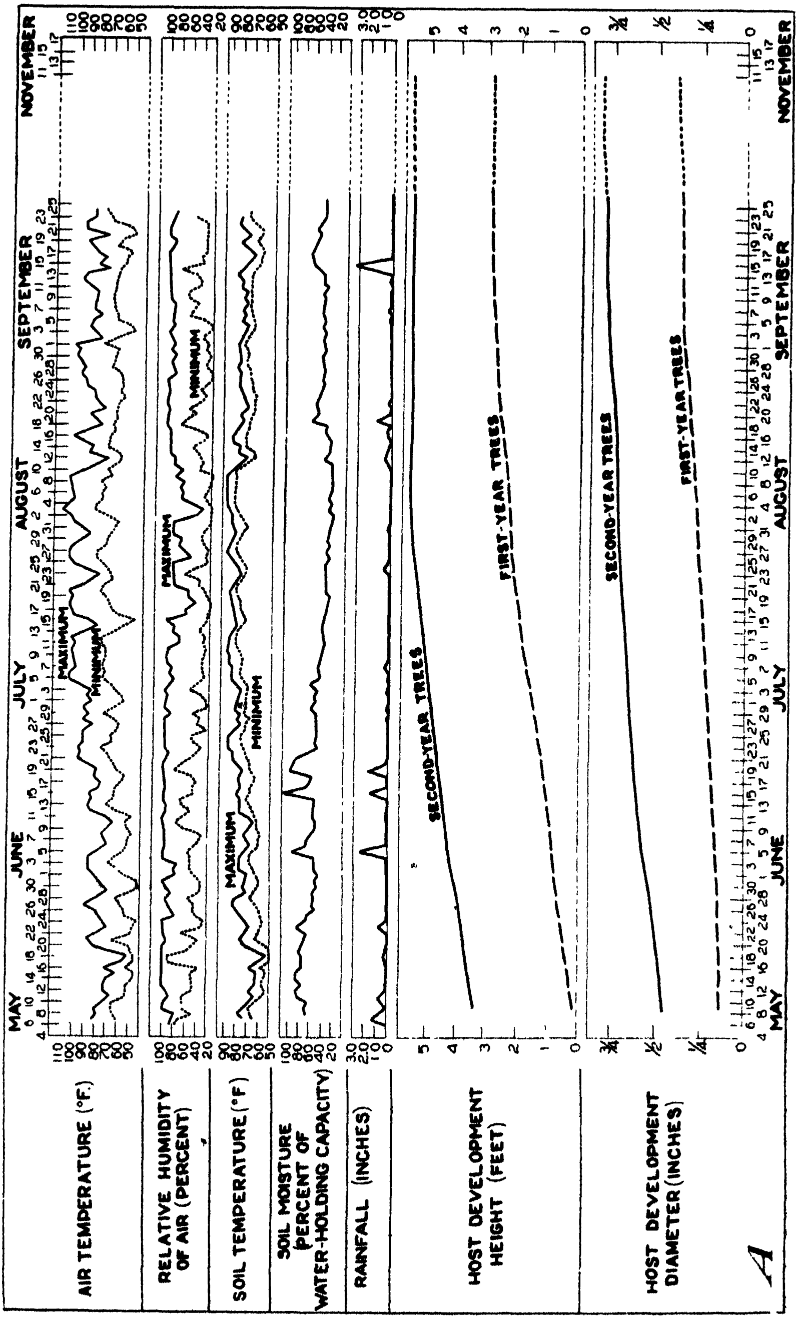


FIGURE 1.—Summary of data relating to seasonal development in 1929 of hairy root and crown gall on nursery apple trees grown from string-wrapped piece-root grafts: *A*, Environmental conditions and tree growth; *B*, incubation periods, natural occurrence of disease, insects present, and seasonal development of hairy root and crown gall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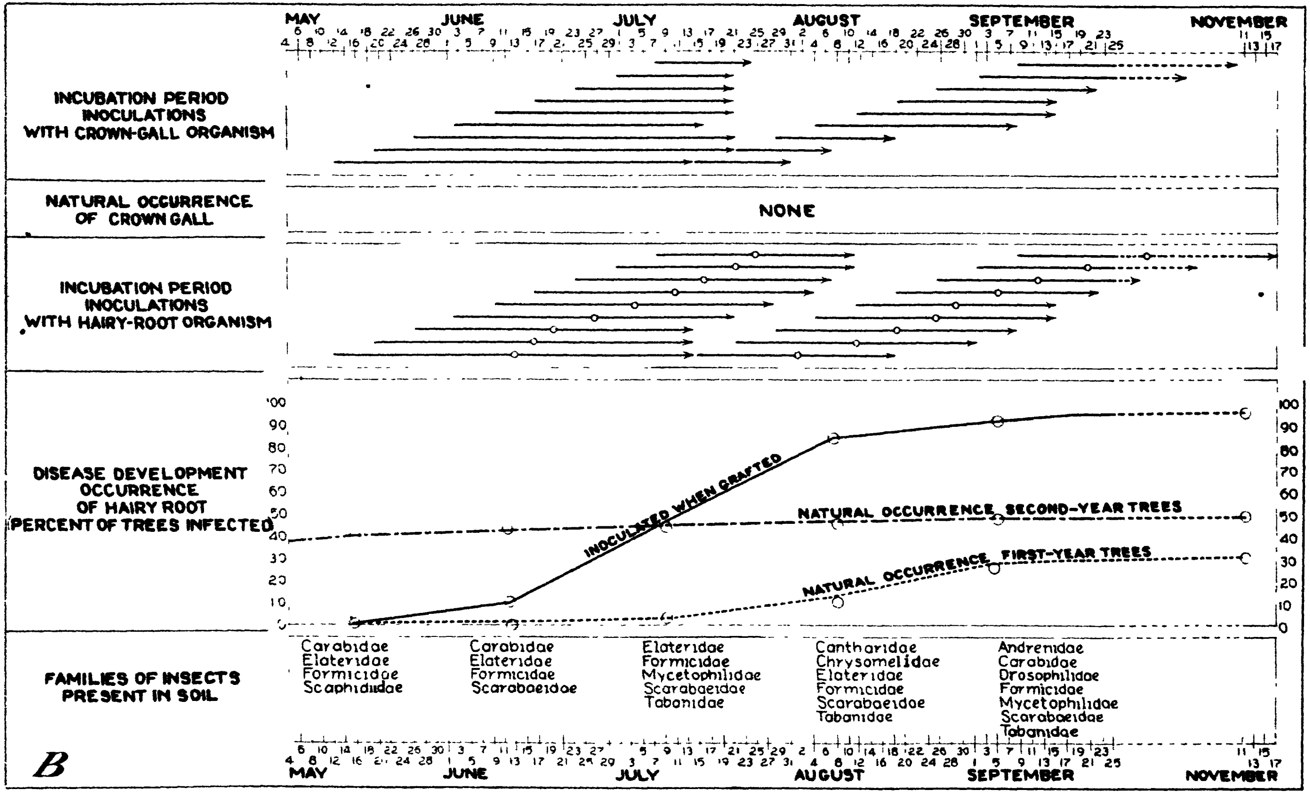
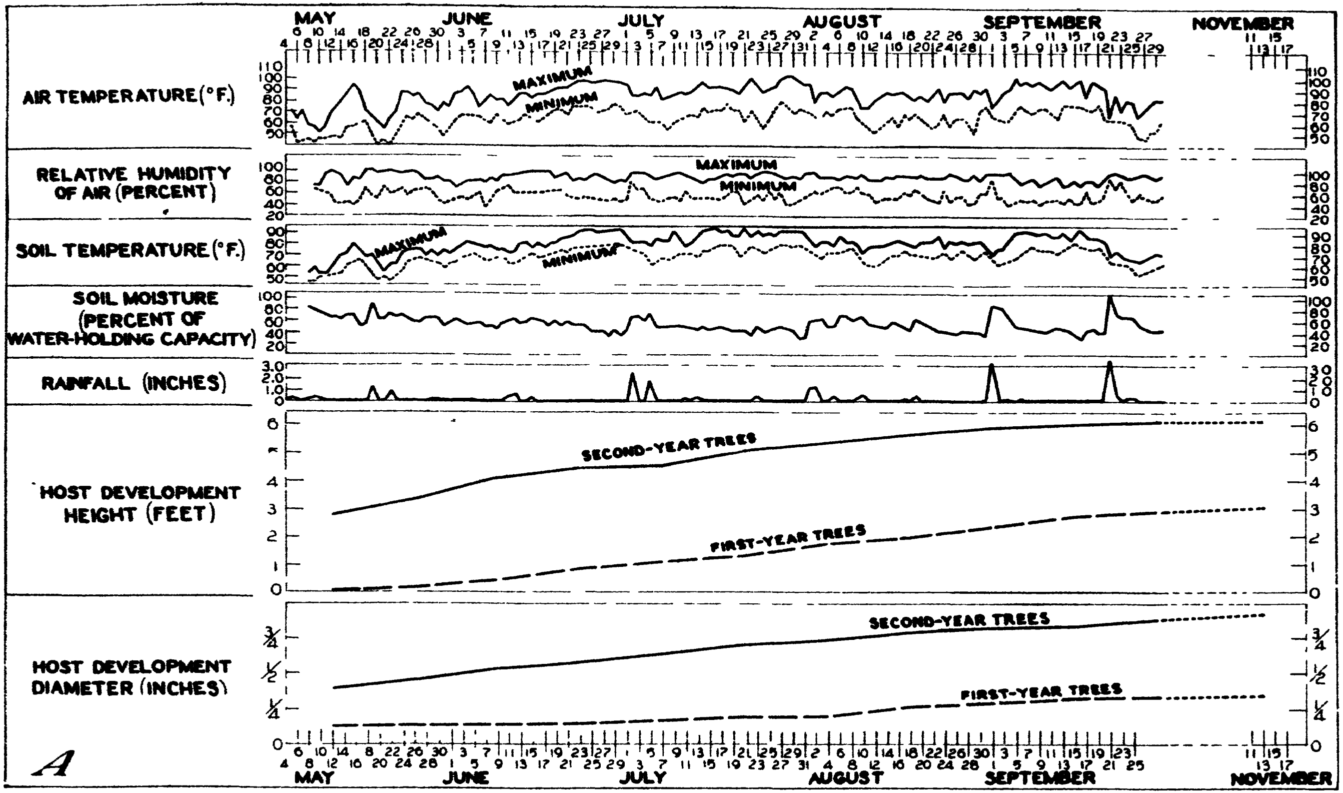


FIGURE 2.—Summary of data relating to seasonal development in 1930 of hairy root and crown gall on nursery apple trees grown from string-wrapped piece-root grafts. A, Environmental conditions and tree growth; B, incubation periods, natural occurrence of disease, insects present, and seasonal development of hairy root and crown gall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HOST DEVELOPMENT HEIGHT (FEET)
HOST DEVELOPMENT DIAMETER (INCHES)
MAXIMUM
MINIMUM
SECOND-YEAR TREES
FIRST-YEAR TREES
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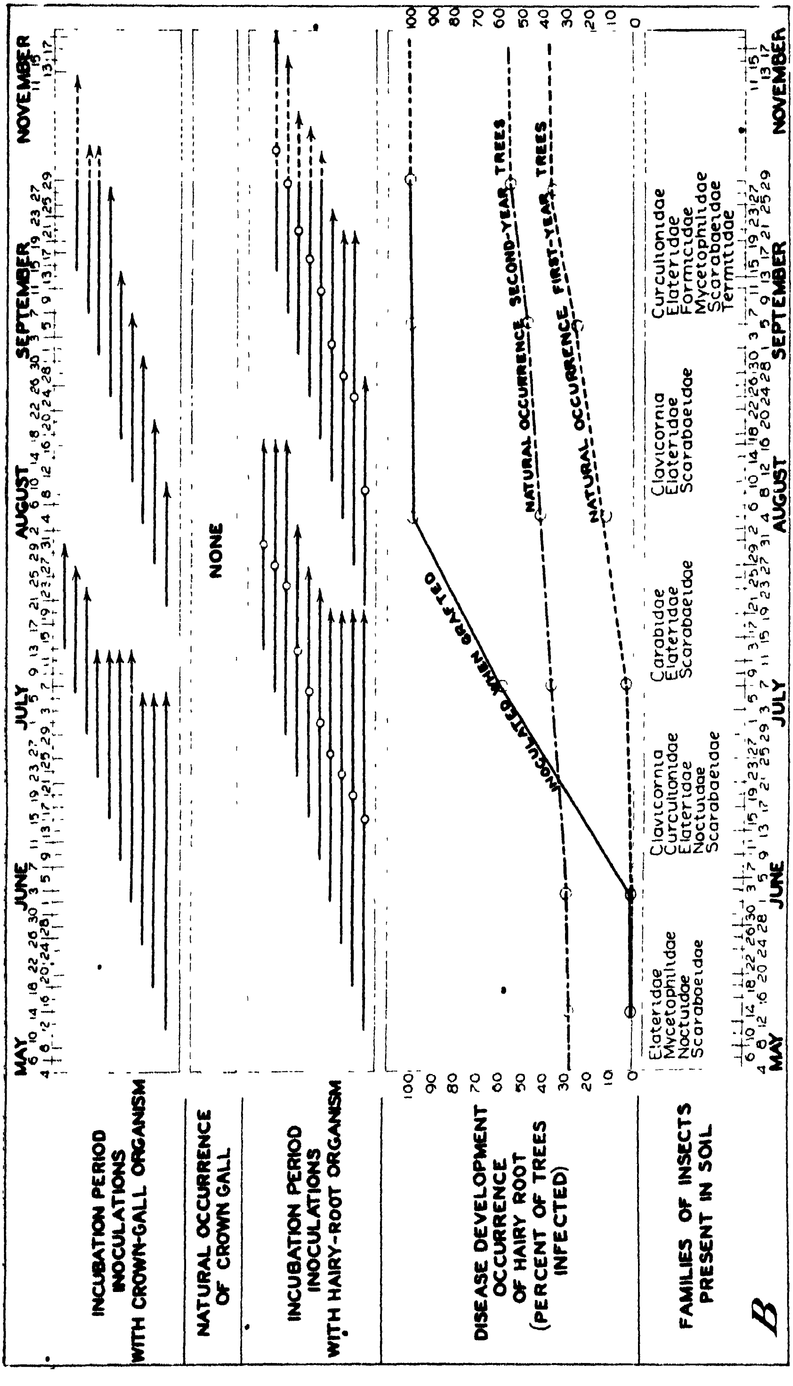

FIGURE 3.—Summary of data relating to seasonal development in 1931 of hairy root and crown gall on nursery apple trees grown from string-wrapped piece-root grafts *A*, Environmental conditions and tree growth *B*, incubation periods, natural occurrence of disease, insects present, and seasonal development of hairy root and crown gall

wrapped grafts. The results are recorded in figures 2, 3, and 5. The average time at which hairy-root symptoms (i.e. roots at least 1 cm long) developed in the string-wrapped grafts appeared to be the early part of July in both 1930 and 1931. The minimum was early in June. The maximum was difficult to determine because of the occurrence of natural infection, which complicated the results. Most of the grafts showed infection by early August. As judged from other inoculations, the maximum probably did not greatly exceed this.

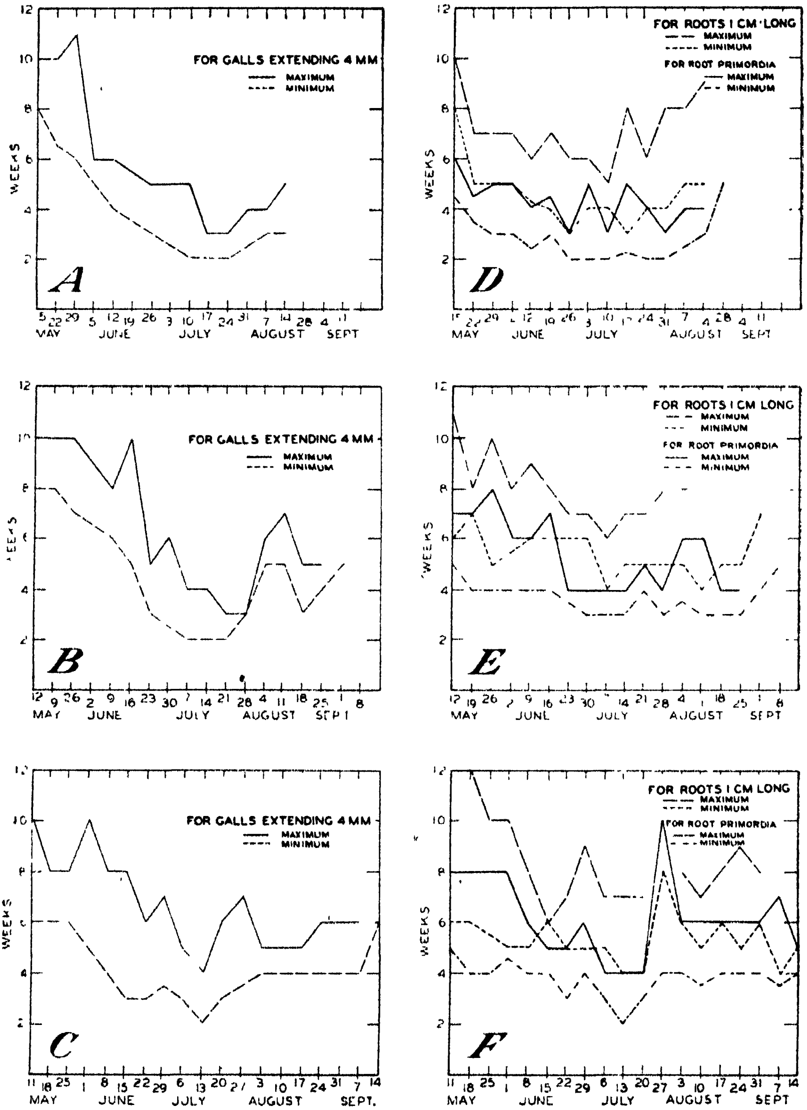

FIGURE 4.—Progressive variations in the length of incubation periods following inoculations made, on the dates indicated, with crown-gall and hairy-root bacteria during the active growing seasons of 1929, 1930, and 1931. The characters of the overgrowths indicating a positive reaction are explained in the text: *A*, Crown gall, 1929; *B*, crown gall, 1930; *C*, crown gall, 1931; *D*, hairy root, 1929; *E*, hairy root, 1930; *F*, hairy root, 1931.

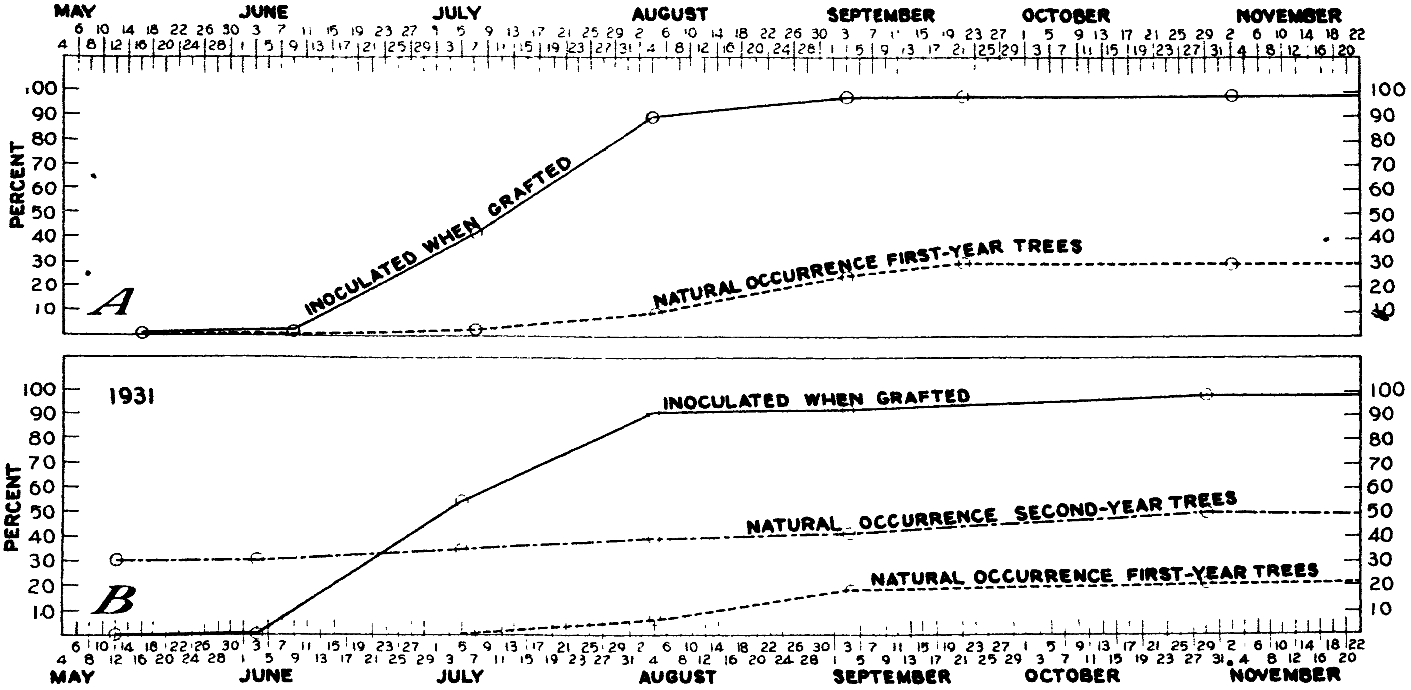

FIGURE 5.—Summary of data relating to hairy-root development in 1930 (*A*) and 1931 (*B*) on nursery apple trees grown from tape-wrapped piece-root grafts

The occurrence of hairy-root symptoms, following inoculations at the time the tape-wrapped grafts were made, was studied in parallel series. In 1930 only 2 percent of the inoculated grafts had shown symptoms by the middle of June. Hairy root was noted on 41 percent of the grafts on July 9, on 90 percent early in August, and on 97 percent early in September. In 1931 none of the grafts showed symptoms when examined early in June. Early in July, 54 percent showed hairy root; early in August there was 91 percent; and by the end of the season there was 99 percent. Like the string-wrapped grafts, the tape-wrapped grafts that were inoculated when made showed hairy-root symptoms early, during the season of long incubation periods, whereas symptoms of hairy root resulting from natural infection appeared later, during the season of relatively short incubation periods. The tape wrapping had little if any influence in preventing infection when inoculations were made at grafting time. Little if any correlation was noted between the time of the occurrence of hairy root following inoculation at grafting and the time of the natural occurrence of the disease.

The strength of the tape wrappers was found to diminish gradually, until the cloth broke under the slightest strain. Where the wrapping was overlapped the cloth lasted much longer. Frequently the tape wrappings had lost their strength by June, so that the hairy-root developments from inoculations easily came through. Sooner or later the wrappings were cracked open by the growth of the trees. This process began in June and progressed with varying rapidity, depending upon such factors as the moisture and temperature of the soil, the amount of overlapping of the wrapper, and the increase in diameter of the tree. It is clear that while the wrapper remained intact, the union was protected against root-chewing insects.

APPEARANCE OF OVERGROWTHS AT DIFFERENT STAGES OF DEVELOPMENT

Hairy root, crown gall, mixtures of these two, and callus or wound overgrowth were studied as they developed after inoculations or special treatments made at different times. The results of these extended studies conform in general with those obtained in limited trials by Riker et al. (*9*). Since a complete new series of tests was started each week during three active growing seasons, the volume of material available was large. Consequently the development of only one representative series for each type of overgrowth is considered here.

The symptoms of the different enlargements in the various stages of development are illustrated in figures 6 to 8. The descriptions given are of typical symptoms that followed the different treatments administered during the latter part of May.

HAIRY ROOT

Hairy-root development is described first for one of the series made during 1930. The cultures and methods of inoculation employed have already been given. The development of the various symptoms was more rapid on the Yellow Transparent trees in Kansas than on Wealthy trees in Wisconsin (*9*).

After 1 week the scalpel cuts in which the inoculations were made showed practically no change from the control scalpel cuts. A slight

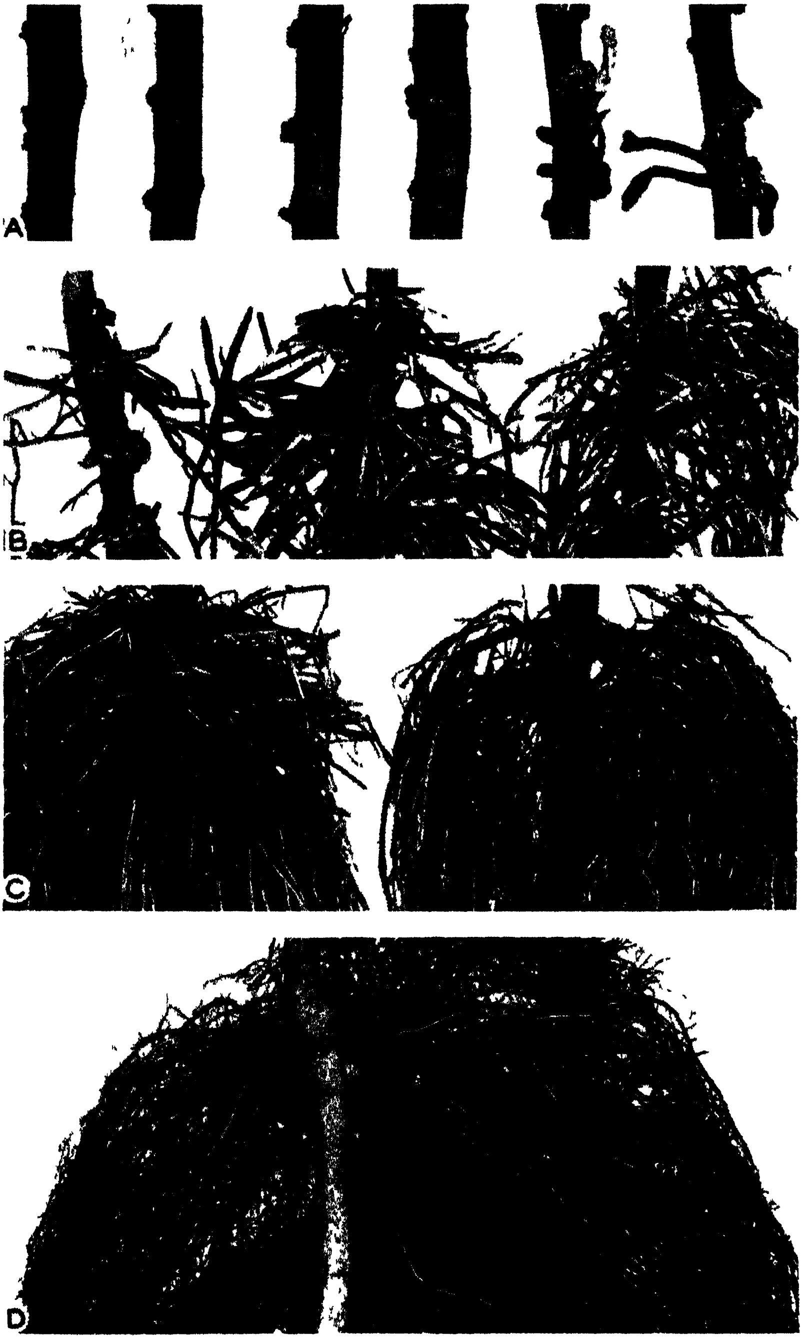

FIGURE 6. Hairy-root symptoms after wound inoculations, as they appeared during the first year on scion wood below ground *A*, After 1, 2, 3, 4, 5, and 6 weeks, respectively, from left to right, *B*, after 8, 10, and 12 weeks, *C*, after 16 and 18 weeks, *D*, after 24 weeks

formation of wound tissue was apparent, especially about the exposed portions of cambium.

After 2 weeks the frosty calluslike tissue had so increased in size that it practically covered the injury. Small hemispherical frosty knobs of new tissue appeared sometimes to extend from the callus. No difference was noticed between the inoculated and control wounds. The lateral extension of the tissue was approximately 2mm.

After 3 weeks the hairy-root tissue had grown until it had an average lateral extension of about 3mm. This was somewhat greater than the callus at the control wounds. At this time small, more or less round protuberances of tissue, root primordia, approximately 2 mm in diameter, made their appearance from the surface of the basal tissue.. Often, but not always, these appeared to be further developments of the knobs mentioned earlier. This stage is indicated by circles in the arrows of the charts designating incubation periods (figs. 1, 2, and 3).

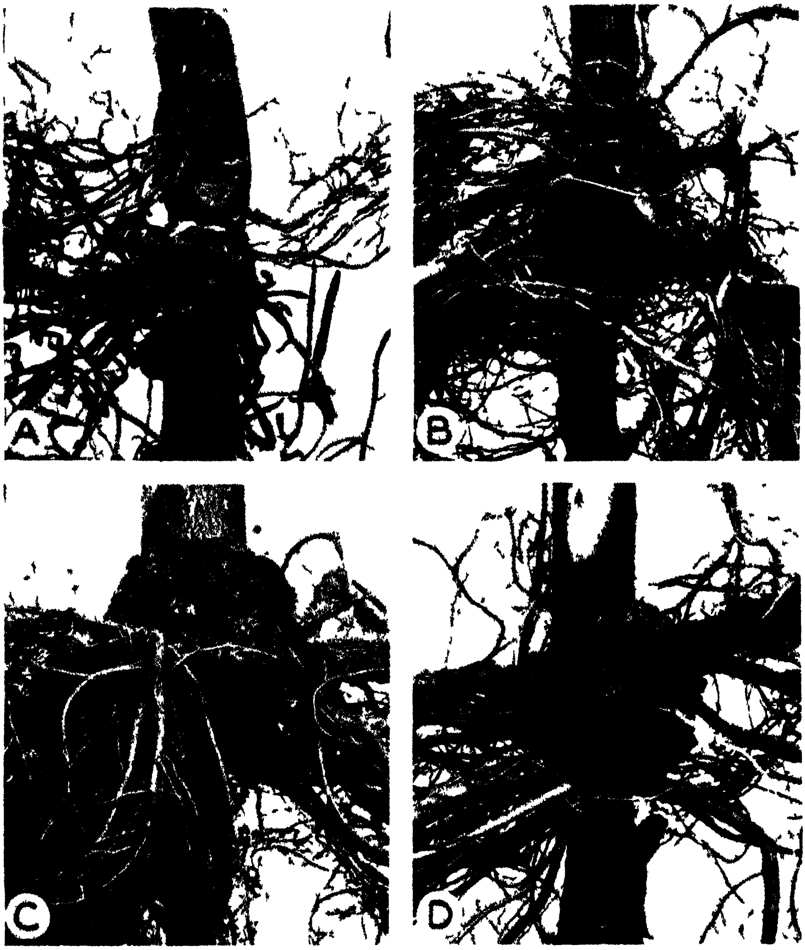

FIGURE 7.—Hairy-root symptoms after wound inoculations, as they appeared during the second year on scion wood below ground: *A*, In May. Many of the smaller roots were killed by frost., *B*, In August. Certain of the roots appear much larger than others. *C* and *D*, In October. Wound-overgrowth tissue appears in conjunction with that of hairy root.

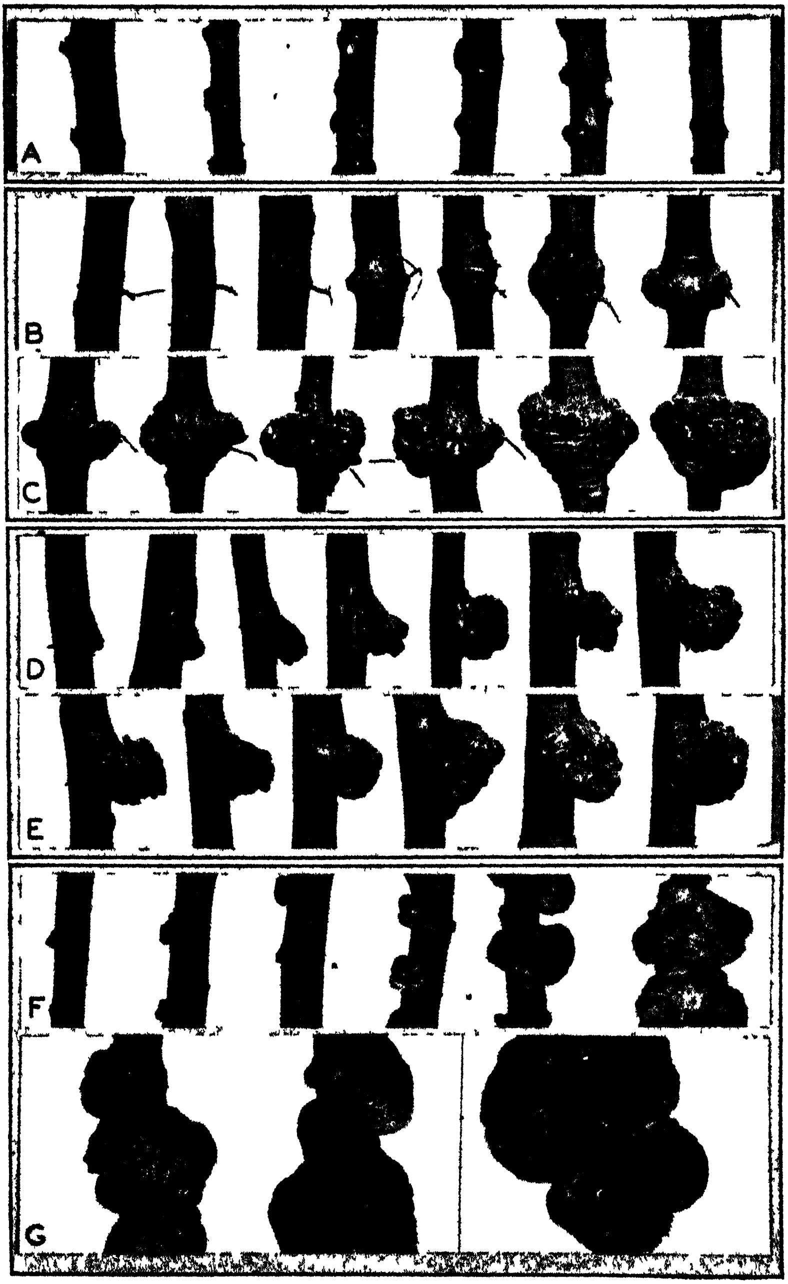

FIGURE 8.—Several types of overgrowths after various treatments as they appeared during the first year on scion wood below ground. *A*, Wounds like those used for inoculation, except that no bacteria were employed, after 1, 2, 3, 4, 5, and 6 weeks, respectively, from left to right. *B*, induced by wire girdles after 1, 2, 3, 4, 5, 6, and 8 weeks, respectively, from left to right, and (*C*) after 10, 12, 14, 16, 18, and 20 weeks. *D*, induced by knife cuts into which were inserted bits of adhesive tape after 1, 2, 3, 4, 5, 6, and 8 weeks, respectively, from left to right, and (*E*) after 10, 12, 14, 16, 18, and 20 weeks. *F*, crown galls that followed wound inoculations as they appeared after 1, 2, 3, 4, 5, and 6 weeks, respectively, from left to right, and (*G*) after 8, 10, and 12 weeks.

After 4 weeks both the basal tissue and the protuberances had enlarged until the average lateral extension was approximately 4 mm. Some of the protuberances had taken on more definitely the appearance of root primordia. In exceptional cases small fleshy roots were starting.

After 5 weeks the basal enlargements had increased a little and root primordia were quite common. Small fleshy roots were also more frequent. The root primordia and roots gave the surface a rough and irregular appearance, in contrast with the relatively smooth surface of crown galls. Some of the small roots were over 1 cm long, indicating the final stage of the incubation period.

After 6 weeks the number of fleshy roots was decidedly greater, and the older ones had reached a length of 2 cm. After this time the basal enlargements increased in size very slowly and some became more or less completely covered by the root developments.

After 8 weeks the fleshy roots had increased considerably both in number and in length. Many had begun to branch, and a few small fibrous roots were found.

After longer periods during the first season following inoculation there was a progressive increase in the number and length of the hairy roots. Large masses of both fleshy and fibrous roots were very common at the end of the growing season. The basal enlargements were ordinarily rather inconspicuous the first year and were well covered with root developments.

The amount of moisture in the soil appeared to be an important factor in the development of the roots. Inoculations made aboveground or in the upper portions of the soil, where it frequently became dried out, showed little or no root development. Evidence was frequently found that roots had started but had been killed by subsequent drying of the surface soil.

The vigor of the trees likewise appeared to influence the development of the hairy-root symptoms. Grafts which had not become well established and had not produced new growth at least a foot high made little or no response to hairy-root inoculations. The relation between the size of tree and the incidence of hairy root at digging time has been more fully discussed by Hildebrand (*4*).

The period in the growing season when the inoculations were made likewise influenced the rapidity with which the symptoms developed. The incubation periods for different stages of the disease were shorter during the summer than in the spring or fall. In some cases when inoculations were made in September, no symptoms were observed until the following year. Although the time at which they appeared varied considerably, the sequence of symptoms remained the same.

During later development after inoculation the hairy-root overgrowths showed a somewhat different character (fig. 7). The roots that were actively growing when cold weather set in and the ground became frozen were killed. However, those that had reached sufficient maturity were able to withstand the winter and to continue their activity the second year. By June of the second year, certain of the larger roots had grown considerably, both in length and diameter. Many small lateral roots appeared that were more likely to be fibrous than fleshy. However, fleshy roots from the basal tissue were quite common.

The basal enlargements likewise had changed somewhat in character. They had grown considerably and had taken on the rather characteristic deeply convoluted type of growth. These various convolutions were built up for several layers and not infrequently particles of soil were incorporated for some distance within the tissue. This type of growth is illustrated in figure 7.

By the middle and latter parts of the second season both the basal enlargements and the hairy roots had increased markedly in size. In all the specimens examined the large lateral roots which developed as part of the hairy-root overgrowth seemed to have established definite connection with the main stem and to be functioning in a somewhat normal capacity.

Nonparasitic tissue of the wound-overgrowth type not infrequently appeared in association with the basal enlargements of the hairy-root development. This seemed to occur in part as a reaction to the interruption in the downward flow of elaborated food in the stem. Apparently the hairy-root development had something of a wounding or girdling action upon the stem, which resulted in the formation of wound-overgrowth tissue (fig. 8) in combination with the hairy-root tissue. All different stages of combination between wound overgrowth and hairy root seemed to occur. Doubtless in some cases the wound-overgrowth tissue occurred as a result of the girdling action of the hairy-root tissue, whereas in other cases formations which were wound overgrowths at the beginning became infected and hairy-root tissue subsequently developed.

CROWN GALL

Studies of crown gall were made similar to those of hairy root. After 2 weeks the reactions to inoculations were similar to those secured from the inoculations with the hairy-root organism. Frosty hemispherical knobs appeared similar to those of hairy root. However, the knobs on crown gall were not observed to develop further. After 3 weeks the lateral extension of the crown gall was about 1 mm greater than that of hairy root and the surface was lobed and comparatively smooth. After 4 weeks the crown galls had increased considerably in size and often showed a lateral extension of 5 to 8 mm. No root primordia were observed.

After longer periods the basic characters of the crown galls did not change, although the formations increased greatly in size as the weeks passed. No roots have been found growing directly from the crown-gall tissue and only infrequently from the main stems of the Yellow Transparent apples near the gall tissue. The sequence of development of the crown galls is shown in figure 8, *F*, *G*. The influence of temperature and moisture on the development of crown gall under controlled conditions has been reported by Riker (*7*).

MIXTURES OF CROWN GALL AND HAIRY ROOT

Parallel inoculations were made with mixtures of the crown-gall and hairy-root bacteria. The resulting overgrowths developed along the lines previously mentioned for crown gall and hairy root and showed all gradations between those two types of formations. The time of the growing season seemed to exert some influence in determining which type of development would appear first and predominate. The ascendancy of the hairy-root organism was greatest comparatively early or late in the growing season, when the temperature was somewhat low. On the other hand, in approximately the middle of the growing season, when the temperature was somewhat high, the crown-gall organism appeared to have the advantage.

WOUND AND CALLUS OVERGROWTHS

The control wounds that were made in conjunction with the inoculations for crown gall and hairy root produced only slight reactions. For the first 2 weeks there was practically no difference between the control injuries and those inoculated with the two organisms (fig. 8). However, after 3 weeks the callus formations which appeared began to decrease in size as the process of healing progressed; by the fourth week the soft outer tissue had begun to slough off; after 5 or 6 weeks the characteristic wound tissue had formed and the development of these wound reactions ceased. Natural infection of the control wounds was rare.

Parallel studies were made upon wound overgrowths induced by girdling with wire. As already stated, aluminum-alloy wire was wrapped twice around the stem and then fastened in order to determine what the reaction of the host plant would be to this interruption in the downward passage of elaborated food. After 1 week there was practically no change, but after 2 weeks the wire wrapping was very tight about the bark (fig. 8, *B*, *C*). After 3 weeks it seemed to be cutting into the bark tissue. After 4 weeks a swelling appeared above the wire that in some cases was sufficient almost to hide it from view. After 5 weeks this wound tissue had increased to a lateral extension of 2 to 4 mm and the wire was almost completely hidden. After 6 weeks the growth had increased in size and after 8 weeks it had a lateral extension of approximately 7 mm around most of the stem. These nonparasitic overgrowth developments so increased in size as the season progressed that at the end of the growing season their diameter was several times that of the main stem. The surface of this tissue had more or less broad undulations very different from the comparatively smooth lobes of the crown-gall tissue or the deep convolutions of the hairy-root tissue. There was a definite cortex. This type of tissue resembled more closely the tissue of hairy root than that of crown gall. As already stated, complete intergradations were found between the surface characters of this type of development and those of hairy root.

Similar wound overgrowths developed after certain knife injuries made on the underground parts of apple stems, about an inch above the union, by an upward cut through one third to one half of the diameter of the stem. A small piece of adhesive tape was inserted under the tissue thus cut in order to prevent its direct healing and to simulate conditions at the lower ends of scions that failed at the tips to make union with the roots. Trees without the tape barriers in the cut usually healed normally in a few weeks without the development of excessive callus. In trees having tape barriers, a small callus had formed on the lower tip of the cut tissue after 1 week (fig. 8, *D*). Within 2 weeks the callus had grown considerably and after 3 weeks it had a lateral extension of several millimeters. The increase in size continued until characters of wound overgrowth appeared similar to those induced by wire girdles. At the end of the season overgrowths of this type had a lateral extension from 1 to 3 times greater than the diameter of the main stem and showed the characters of wound overgrowth as already described.

These studies on various overgrowths of known origin were very helpful in the diagnoses of overgrowths that developed under natural conditions.

DATE OF NATURAL INFECTION

The natural occurrence of the various overgrowths in this graft-knot complex was recorded at stated intervals throughout three growing seasons. No natural occurrence of crown gall was found among the trees under observation. In other plantings in this locality where a large number of trees were observed, approximately 0.1 percent of the trees were affected with crown gall. The early stages of excess callus or wound overgrowths appeared from time to time. Some of these were grown over as the trees developed (*12*), and some, like the girdle or wound knots (fig. 8), continued to develop; but in this particular locality most of them sooner or later became infected by hairy-root bacteria, and their classification was changed to hairy root. Hildebrand (*4*) has recorded the activity of root-chewing insects in opening infection courts in such tissue. Since the location of this work was made largely on the basis of the severity of the hairy-root infection, this result was expected.

The natural occurrence of hairy root in 1929 on first-year apple trees grown from string-wrapped grafts was recorded at weekly intervals (fig. 1). Natural infection appeared slowly until the end of June, when 11 percent of the trees showed symptoms of disease. By the end of July practically no increase had occurred. However, by the end of August, 28 percent of the trees showed infection; and by the end of September, 38 percent. After this time, apparently correlated with lower temperature and perhaps cessation of growth by the host plant, there was comparatively little increase until the return of warm weather. During the second year (1930), on the same trees (fig. 2), there was a progressive increase up to 49 percent in November, when the trees were removed.

In 1930 the natural occurrence of hairy root on first-year trees (fig. 2) grown from string-wrapped grafts was quite similar to that in 1929. There was 4 percent infection early in July, 13 percent early in August, and 27 percent early in September. There was relatively little increase between that time and the middle of November, when there was 30 percent. During the following year (1931; fig. 3) on the same trees, that were second-year trees by this time, there was practically no development until June. By early July there was an increase to 37 percent, by early August to 42 percent, by early September to 48 percent, and by late October to 57 percent.

In 1931 the natural occurrence of hairy root on first-year trees (fig. 3) grown from string-wrapped grafts was very similar to that of the previous year. Only 3 percent appeared early in July. Early in August there was 14 percent; early in September, 26 percent; and late in October, 38 percent.

Similar results were secured for tape-wrapped grafts (fig. 5). However, the appearance of symptoms was delayed somewhat by the tape wrapping.

The natural occurrence of hairy root in 1930 on trees grown from tape-wrapped grafts was recorded as before. The first hairy-root symptoms appeared early in July, when 2 percent of the trees showed hairy root. Early in August, 9 percent showed hairy root; early in September, 25 percent; and late in September, 30 percent. The next year (1931) the number of trees infected rose gradually to 50 percent.

In 1931 the natural occurrence of hairy root on tape-wrapped grafts began with 6 percent early in August. Early in September there was 18 percent, and late in October 21 percent.

The date of natural occurrence of most of the knots on tape-wrapped grafts appeared to be correlated with that of knots on string-wrapped grafts. The date of natural occurrence of knots on both types of grafts seemed correlated with warm weather, active growth of the nursery trees, short incubation periods, decreased protection of the unions by wrappers, and insect activity.

The average date of natural occurrence of an infection appearing on any particular date may be determined by subtracting the number of days of the average incubation period from the date of appearance. For example, to determine the average date of natural infection for new developments that appeared in first-season trees on September 3, 1930, one observes (fig. 2) that the average incubation period ending September 1 began July 21. Therefore, the new infection showing September 3 came from infections taking place about July 21.

The foregoing data indicate that in the plantings under observation most of the natural occurrence of hairy root was not the result of infection induced at grafting time, but was due to certain factors that began to function usually in June after the grafts were planted.

DISCUSSION

The evidence presented in the preceding pages has a definite bearing upon the consideration of control measures. The studies were made in a place where infectious hairy root was prevalent and consequently deal with severe rather than with average conditions. Since nearly all the bacterial overgrowths on the underground parts of nursery apple trees occur at the union, it has been commonly considered that infection takes place approximately at the time the grafts are made. Various antiseptics are therefore applied to the seedlings and to the grafts. Such treatments by the writers have been relatively unsuccessful in this region. Wrappers of adhesive tape have been less efficient for control here than in most places. Apparently, therefore, the chances of developing control methods would be enhanced by determining the time and the conditions under which natural infection takes place.

From the data presented in figures 1 to 5, only a small part of the hairy-root infection naturally occurring appeared to be initiated at grafting time. However, this small part may be important as a potential source of inoculum for subsequent infections.

The date of natural infection for most of the hairy root was apparently correlated with the occurrence of warm weather, rapid growth of the trees, activity of soil insects, relatively short incubation periods, and reduced protection from the wrappers employed. For first-year trees this date was during the middle and latter part of the growing season. New infections continued to occur during the second growing season. In other regions, where the trees stood longer in the nursery row and where hairy root was serious, new infections appeared in the third and fourth seasons (*12*).

The possibility of very long incubation periods perhaps needs further consideration. Relatively dormant trees have been found to carry the hairy-root bacteria for long periods and to show no symptoms until active growth was resumed. The problem to be considered is whether small amounts of inoculum at grafting time might remain dormant long enough to account for the occurrence of hairy root late in the first season and perhaps in the second, third, and fourth season. Although this possibility cannot be easily disproved, the following lines of evidence show that if such a condition occurs it is very unusual: (1) Siegler and Piper (*13*) have reported that bacteria inoculated into aerial parts of trees could be reisolated rarely if at all after 65 days; (2) most of the inoculations made at grafting time have yielded results within ordinary incubation periods and the few infections that appeared later were probably caused by natural infection rather than by the inoculations; (3) the studies on incubation periods showed definite maxima beyond which no symptoms appeared; (4) dormant trees that harbored the bacteria after inoculation in the fall showed symptoms promptly, if at all, when active growth was resumed; (5) the phenomena observed are easily explained by other well-defined factors. It appears, therefore, that the evidence at present available does not justify consideration of common incubation periods as continuing long enough after the date of grafting to account for the date on which the greatest part of the natural infection appeared.

Another phase of the complex hairy-root problem which has been at least partly explained is the failure in this locality of the writers' antiseptic treatments and the lower efficiency of adhesive-tape wrappers. Since root-chewing insects seem implicated during the growing season as important factors in opening infection courts, doubtless the present control measures against graft knots may be supplemented by others that take into account the newly determined date of the natural occurrence of infection.

SUMMARY

The seasonal development of crown gall, hairy root, and wound overgrowth has been followed through the growing seasons of 1929, 1930, and 1931 in Kansas. Crown gall and hairy root were induced by inoculation with single-cell cultures of the causal organisms. Wound overgrowth was induced by wire girdles and by knife cuts. Successive stages in the development of all these overgrowths are described and illustrated.

The following records were kept: Air temperature and humidity, soil temperature and moisture, rainfall, development in height and diameter of both first- and second-season nursery apple trees, incubation periods of both the crown-gall and the hairy-root bacteria, occurrence of hairy root following inoculation and under natural conditions, and groups of insects prevalent in the soil. During this study different seasons have been relatively wet, dry, or intermediate.

The incubation periods for crown gall and hairy root have been relatively long in the spring and fall and relatively short in the summer. They were apparently correlated with temperature and with active growth of the trees.

Both crown gall and hairy root developed after suitable incubation periods following inoculations made at grafting time. Adhesive tape wrappers delayed the appearance of symptoms slightly but did not reduce the number of infections which appeared.

Mixtures of various overgrowths appeared, especially on the second-year trees.

The evidence available points to soil insects, including white grubs, wireworms, and fungus gnats, as important agents in opening infection courts for bacteria during the growing season.

Although a small amount of the natural occurrence of hairy root was traced to infection at the time of grafting and was doubtless important as a source of inoculum, most of it seemed to follow natural infection during the middle and latter part of the first growing season and during the second growing season. Much of the natural infection appeared to be correlated with warm weather, active growth of the nursery trees, short incubation periods, decreased protection of the unions by wrappers, and insect activity.

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HAIRY ROOT, CROWN GALL, AND OTHER MALFORMATIONS AT THE UNIONS OF PIECE-ROOT-GRAFTED APPLE TREES AND THEIR CONTROL[1]

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INTRODUCTION

The control of hairy root (*Phytomonas rhizogenes* Riker et al.), crown gall (*P. tumefaciens* (Smith and Town.) Bergey et al.), wound overgrowth, and perhaps other enlargements that occur at the unions between scion and root on nursery apple trees grown from piece-root grafts has been attempted in a number of different ways. These attempts have been stimulated by the fact that very commonly the susceptible varieties of nursery trees have shown these enlargements to a serious extent at digging time. In some extreme cases practically a whole planting has been lost. The prevalence of these diseases has been largely responsible for a change in the method of propagation followed by many of the nurserymen east of the Mississippi River. In this region apple trees are propagated commonly by budding, a method which largely obviates the problem of enlargements at the union and which may give a better stand. However, this method of propagation is reported to be more expensive, to require labor during a very busy part of the growing season, and to provide nursery trees on seedling roots. These factors have caused many of the apple-tree growers in the Middle West to continue piece-root grafting despite the loss from these diseases. To find a means of reducing the loss has been the object of the present studies. Several preliminary accounts on certain phases of this work have already appeared (*23*, *25*, *28*, *32*, *33*).[2]

ECONOMIC IMPORTANCE

The actual harm done to the nursery apple trees by enlargements at the union has been discussed by several writers, including Stewart (*45*) and Jakovlev (*13*). However, much of the work reported on the injury caused by graft knots[3] is open to question because of the inadequate diagnosis of the malformations studied. As discussed later, the several different kinds of graft knots that occur doubtless produce various effects upon the trees. More evidence is needed on this question of injury. Since the differentiation of certain kinds of graft knots has been accomplished (*18*, *19*, *26*, *27*) the influence of hairy root on the growth of the trees has received some attention. Comparatively little evidence, beyond that already noted by Riker

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[2] Reference is made by number (italic) to Literature Cited, p. 937

[3] A general term for any overgrowths at the union.

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and Keitt (*31*), has been secured as yet by the present writers on the influence of crown gall and wound overgrowth of known identity on the growth of nursery trees.

Some data on the effect of hairy-root infection on the growth of nursery apple trees were accumulated in 1929 and 1930 (*27*). Second-season trees of the Yellow Transparent variety were employed. Smooth trees and corresponding hairy-root trees that had become naturally infected the previous season were chosen for measurements. On May 17, 1929, the average height and diameter of 100 smooth trees were, respectively, 32.1 and 0.37 inches. Measurements were made of the same trees September 17, 1929. A few of the healthy trees had become infected during the season and were omitted from the final results. The average height and diameter of the smooth trees were, respectively, 51.8 and 0.59 inches; those of the hairy-root trees were 49.2 and 0.56 inches. These averages show a slight advantage in growth during the season, of 2.6 inches in height and 0.03 of an inch in diameter, in favor of the smooth trees. Repetitions of these trials during the season of 1930 also showed a slight advantage, 5.1 inches in height and 0.08 of an inch in diameter, in favor of the smooth trees. These results are in general accord with those of various writers, including Fracker (*6*), Swingle and Morris (*47*), and Muncie (*18*); the last-named reported a reduction in water-flow through woody knots. However, the data presented in the present paper are so limited and the variations among corresponding trees so great that the differences found seem to have little if any significance. These data, however, raise the question whether the hairy root may perhaps be slightly detrimental to the tree.

In an effort to discover whether the hairy roots were able to function as ordinary roots, a rather severe test was made. On May 17, 1929, 100 hairy-root trees that had become infected following inoculation the previous season were treated. The soil was removed so as to expose the main stem of the tree with as little disturbance as possible to the hairy-root development. The stem was then cut off from the main root system just below the hairy-root development, and the soil was replaced. Ninety-five of the trees thus treated lived throughout the season. By September 17, 1929, they had increased, on an average, 4.5 inches in height and 0.05 of an inch in diameter. Similar trials with like numbers of artificially infected trees in 1930 showed almost identical results, only 5 percent of the trees dying. From May 20 to September 20, 1930, the trees increased, on an average, 6.6 inches in height and 0.12 of an inch in diameter. Smooth trees received similar cuts in corresponding positions. This left them with no roots at all, and of course they died promptly. The fact that the trees supported only by hairy-root developments were able to live shows that these roots have certain functional capacities.

The experiments described in the two preceding paragraphs were made on trees that were grown under comparable conditions in adjacent rows in the nursery. It appears, therefore, that trees which were deprived of all roots except the hairy roots made much less growth than untreated smooth trees or trees that had both natural and hairy roots.

In limited trials following pure-culture inoculations Riker and Keitt (*31*) have found crown gall distinctly detrimental to small nursery apple trees.

Whatever may be the actual influence on the apple trees of the various enlargements at the union, it does not lessen the scope of the problem of control. The problem of these enlargements of trees grown from piece-root apple grafts becomes one of prevention.

These malformations vary considerably not only in their external characters but also in their internal structure.

CAUSES AND DESCRIPTIONS OF OVERGROWTHS

Several different kinds of overgrowths occur on nursery apple trees grown from piece-root grafts. Perhaps the most important are: (1) Infectious hairy root, caused by *Phytomonas rhizogenes* (*27*); (2) crown gall caused by *P. tumefaciens* (*10, 43*); and (3) callus or wound overgrowth, which is nonparasitic (*18, 19, 30, 31*). An understanding of the nature of these formations is obviously desirable in making a satisfactory approach to the problem of their control.

Hairy root is the most common enlargement found in some nurseries. It consists usually of a mass of fleshy or fibrous roots that spring from more or less conspicuous enlargements on the main stem. These enlargements seem to have their origin at the union or at some other point of injury through which the bacteria may have gained entrance. The enlargement at the base of the root formation is usually quite hard, owing to irregular masses of woody elements which seem to be directly connected with the vascular system of the main stem. Hairy-root enlargements have been described by various writers, including Hedgcock (*10*), who called them woolly knots to distinguish them from other overgrowths, and recently they have been described in relation to *Phytomonas rhizogenes* by Riker et al. (*27*). These parasitic hairy-root developments should not be confused with other hairy-root formations which apparently are nonparasitic (*18, 24, 31*).

Crown gall occurs as a result of infection by *Phytomonas tumefaciens*. The causal bacteria may supposedly gain entrance to the tissue at the time the graft is made, or through wounds produced at some later period (*18, 31, 38, 41*). The galls proper on apple are comparatively smooth and as a rule do not have roots growing directly from the surface, although roots frequently grow from the main stem around the edge of the gall. Ordinarily the surface of these galls is not covered with a true bark but either with a layer of dead cells or with actively growing gall tissue. The interior is usually soft like cortical tissue but may contain hard woody elements. Galls of this description render infected trees unsalable, but the small percentage of such galls on nursery apple trees in the Middle West makes them of little economic importance. A more complete description of these crown galls has been given by several writers, including Smith et al. (*43*), Riker and Keitt (*31*), and Muncie (*18*).

Callus or wound-overgrowth development begins commonly on the cut surfaces of the scion and root after they have been fitted together and stored for some time in moist packing material. This formation of callus is of course necessary to establish a union between the scion and the root. However, a union may be imperfect because of a variety of influences, such as a poor fit in grafting, a scion larger than the root, loose wrapping, irregular callus development, and the formation of cork over the callus tissue (*10, 18, 31, 35*). In many cases the development of callus may continue over a long period and may result in an enlargement of sufficient size to deform the tree. Enlargements of this character have been stimulated by girdling young trees with wire or with a wrapper that failed to rot in a comparatively short time (*24*, *29*, *30*). They are caused apparently by the blocking of the downward flow of elaborated food by the girdle and the activity of the tissue immediately above the point of restriction. Apparently a similar condition occurs where the union between the scion and the root is relatively imperfect. As explained later, it has appeared from the cause of these malformations that they might be controlled, as pointed out by Hedgcock (*10*), if a better union between scion and root could be secured and the formation of excess callus prevented by carefully fitting the grafted parts and by using suitable wrapping.

Mixtures of these different types of development occur with more or less frequency from time to time. Complete intergradations between them may be found in many nurseries. Likewise, inoculations with mixtures of the crown-gall and hairy-root organisms have given complete intergradations of the crown-gall and hairy-root symptoms (*24*). Perhaps the most common mixture encountered in the average nursery is that of hairy-root and wound overgrowth. These occur either from the infection of wound overgrowths with hairy root or from the formation of overgrowths at hairy-root infections, doubtless in large part because of the same factors that induce overgrowths above mechanical injuries or girdles. The relative frequency of different types of mixtures has been found to vary in different localities.

Although hairy root, crown gall, and wound overgrowths are apparently the more important types of graft knots, other kinds of overgrowths may occur with considerable frequency under certain conditions. Among these may be mentioned (1) burrknots, described by a number of writers, including Birmingham (*1*), Brown (*2*), Swingle (*46*), Hatton et al. (*9*), Carne (*3*), and Siegler and Piper (*40*), the last-named workers producing malformations morphologically identical with their apple strain of bacteria but failing to isolate these bacteria from naturally occurring burrknots; (2) incompatible unions, mentioned by Riker (*22*); and (3) noninfectious hairy root described by Muncie (*18*), Riker et al. (*27*), and others, the cause of which still remains obscure.

Since the relative prevalence of the different kinds of graft knots has a definite bearing upon the types of control measures that should be employed, their distribution in the nursery was examined.

DISTRIBUTION OF OVERGROWTHS IN THE NURSERY

Malformations, particularly at the unions, on trees grown from piece-root apple grafts appear to occur wherever apple trees are propagated by this method. There has been some confusion about the relative distribution of these malformations because of the difficulty of diagnosis. It is only within the last few years that the causal differences in hairy root, crown gall, and callus developments have been noted. In the survey reported by Riker and Keitt (*31*) crown gall was separated from wound overgrowth and hairy root. However, for the most part these writers placed both wound overgrowths and hairy root in the same classification without distinction. Muncie and Suit (*19*) have reported the results of extensive surveys in which they found that infectious hairy root was of comparatively small consequence. In the light of other investigations (*25*, *27*, *37*) where the cause and symptoms of infectious hairy root are more clearly defined, it appears that this disease is of great economic importance, especially under some conditions.

The distribution in the nursery of the various malformations under consideration on the underground parts of nursery apple trees has been studied by the present writers in several different localities. Each tree in the nursery row was examined after being dug, and the relative positions of the smooth and the knotted trees were noted. If an overgrowth was found, its size, position, and character were recorded in detail. Two examples of very common types of occurrence and distribution are given in figures 1 and 2. The actual field data have been so arranged that much about the condition of each tree might be indicated by two letters. A comparatively light incidence of overgrowths often shows the type of distribution illustrated in figure 1. This record was taken in one long continuous row of which the trees recorded comprise only a small part. A common distribution showing a heavy incidence of overgrowths appears in figure 2. Results similar to those just cited (figs. 1 and 2) were secured in most of the studies on control reported later (tables 1 to 5). The details of these records are omitted because of their large volume.

These records, of which figures 1 and 2 are small and condensed examples, suggest that (1) if the hairy-root organism comes from the soil where the grafts are planted it is well distributed there; (2) if it comes in with the grafts it is also well distributed among the grafts; and (3) after the disease develops it may spread somewhat along the nursery row. The question of the source of hairy-root inoculum has received detailed consideration in other papers (*11*, *29*). Crown gall was found only occasionally. In most of the studies reported in this paper it comprised less than 1 percent of the overgrowths. Excess callus or wound overgrowths occurred with relative frequency on the first-year trees; on second-year trees they were often either healed over or infected with hairy-root bacteria; on still older trees they frequently occurred in combination with hairy root.

DISTRIBUTION OF OVERGROWTHS ON TREES

Most of the overgrowths on the underground parts of the main stems of the trees occur at the union between scion and root, as shown in figures 1 and 2. Further records of 54 trials on trees grown from grafts wrapped with string and of 54 corresponding trials on trees wrapped with adhesive tape are shown in table 1. These trials were made in Iowa, Kansas, Minnesota, Missouri, Nebraska, Oklahoma, and Wisconsin on 1- to 4-year-old trees of the following varieties: Bayfield, Black Ben, Delicious, Dudley, Early Harvest, Fameuse, Florence (crab), Gano, Golden Winesap, Goodhue, Jonathan, McIntosh, Northwestern Greening, Oldenburg, Perkins, Red Siberian (crab), Red Wing, Wealthy, Whitney (crab), Yellow Transparent, and York Imperial.

On an average, less than 5 percent of the trees had overgrowths on the scions and less than 2 percent on the roots, whereas approximately 25 percent of the trees grown from commercial string-wrapped grafts, and from 7 to 14 percent of the tape-wrapped trees, had graft knots at the unions. There was considerable variation in individual trials.

○	○	○	○	○	○	○	○	○	○	Ks	Hu	○	○	○
○	○	○	○	○	○	○	○	○	Hu	○	○	○	○	○
○	○	○	Hu	○	○	○	○	○	○	Hu	○	○	○	Hu
○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Ku	○	○	Hu	hu	○	○	○	○	○	○	hc	○	○	○
○	○	○	○	○	Hu	○	kc	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○	Hc	○	○	○
hc	Ks	○	○	Hc	○	○	○	○	○	○	Hc	○	○	○
○	○	Hc	○	○	○	○	○	○	○	○	○	○	Hc	○
○	Ku	Ks	○	○	○	○	○	○	Hu	○	○	○	○	○
Ku	○	○	○	hu	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
○	Ku	○	○	○	○	○	○	○	○	○	○	Ku	○	○
○	○	Hu	○	○	○	○	Hu	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
○	○	○	○	Hc	○	○	Hu	○	○	Hu	○	○	○	○
Hu	○	○	○	○	○	○	○	○	○	○	Hu	○	○	○
○	○	○	○	○	○	○	○	○	Gs	○	○	○	○	○
○	○	○	○	○	○	Hu	Ku	○	○	○	○	○	○	○
○	○	○	○	○	○	○	○	○	Hu	○	○	○	○	○
○	○	○	Hc	○	○	○	○	○	○	○	Hu	Hc	○	Hu
○	○	○	○	○	○	○	○	○	○	○	○	Hu	hc	○
○	○	○	○	Hu	○	○	Ku	○	○	Hu	○	Hu	○	○
Hu	○	○	○	○	○	○	○	○						

FIGURE 1.—Distribution of enlargements in one continuous row (reading from left to right) on 2-year-old Yellow Transparent apple trees propagated in Iowa from tongue grafts. The characters of the individual trees are summarized according to the following symbols: *, Smooth, *G*, large crown gall, *H*, large hairy root, *h*, small hairy root; *K*, large callus or wound overgrowth; *k*, small callus or wound overgrowth; *c*, located on the scion; *u*, located on the union; *s*, located on the stock.

From 0 to 18 percent of the trees had overgrowths on scions, from 0 to 93 percent on unions, and from 0 to 7 percent on roots. These results confirm those of a number of earlier papers. However, the percentages of overgrowths at the union in these trials were lower than those reported by several workers, including Doidge (*4*) and Muncie (*18*). This difference is probably due to recent improvements in grafting technic and in cultural practices.

Some difficulty was experienced in differentiating incipient hairy root from burrknots on the scions of such varieties as Bayfield and

Okabena, on which burrknots occur frequently. Such burrknots have not been included in these records. Overgrowths were found from time to time on the lateral roots. In some cases they appeared to follow mechancial injury incident to cultivation, but more frequently they were in positions where only insects could have reached them. This subject is discussed more fully by Hildebrand (*11*).

Hu	Hu	Hu	O	Hu	Hu	Ks	O	Hu	O	Hu	O	Hu	Hu	O
O	O	Hu	O	O	Hu	hc	Hu	Hu	O	Hu	Hu	Hu	Hu	O
O	Hu	Hu	Hu	O	Hu	O	Hu	O	Hu	O	Hu	O	Hu	Hu
Kc	O	Hu	O	Hu	Hu	Hu	Hu	O	O	Kc	Hu	O	O	Hu
Hu	O	Hu	O	Hu	Hu	O	Hu	Hu	Hu	O	O	O	Hu	Hu
Hu	Hu	Hu	Hu	Hu	Hu	O	O	ku	O	O	kc	kc	Hu	O
O	Hu	O	Hu	O	Hu	Ku	Hu	Hu	Hu	Hu	O	Hu	O	O
Hu	O	Hu	Hu	O	O	Hu	O	Hu	O	Hu	O	Ku	Hu	O
Hu	O	Hu	Hu	Ku	Hu	Hu	O	Hu	Hu	O	O	O	Kc	Ku
Hu	O	Hc	Hu	O	Hu	Hu	O	Hu	O	Hu	O	Hu	O	O
Hu	Hu	Hu	O	Hu	Hu	O	Hu	O	Hu	Hu	O	Hu	O	Ku
Hu	hc	O	Hu	O	Hu	Hu	O	Hu	O	O	Hu	Hu	Ks	Ku
Hu	O	Hu	Hu	hu	Hu	O	O	O	Hu	Hu	Hu	O	Hc	Hu
O	Hu	Hs	Hs	O	O	Hs	O	Hu	Hu	Hu	O	Hu	Hu	Hu
Hu	Hu	O	Hu	Hu	Hu	Hu	Hu	Hu	hu	Hu	Hu	O	Hu	Hu
Hu	Hu	Hu	Hu	O	Hu	Hu	O	Hu	O	Hu	O	Hu	Hu	O
Hu	Hu	O	O	Hu	Hu	Hu	O	Ks	O	Hu	O	O	kc	O
O	hu	hu	hu	Kc	Hu	O	Hu	Hu	Hu	Hu	Hu	Hu	Hu	O
O	O	hu	O	hc	O	O	c	Hu	Hu	Hu	Hu	hc	Hu	Hu
Hu	Hu	O	Hu	Hu	Hu	O	Hu	O	Hu	O	O	Hu	Hu	Hu
O	O	O	kc	Hu	Hu	Hu	Hu	Hu	O	O	Hu	Hu	Hu	Hu
Hu	Hu	O	O	O	Hs	Hu	Hu	hu	O	Ku	Hu	O	Ku	O
Hu	Hu	Hu	Hu	O	Hu	hu	Hu							

FIGURE 2.—Distribution of enlargements in one continuous row (reading from left to right) on 2-year-old Wealthy apple trees propagated in Kansas from tongue grafts. The characters of the individual trees are summarized according to the symbols shown for figure 1

TABLE 1.—*Relative frequency of occurrence of enlargements on various underground parts of nursery apple trees grown from piece-root grafts, 1929–31*

Year	Trials	Wrapper	Trees examined	Trees with enlargements on—		
				Scion	Union	Stock
	Number		*Number*	*Percent*	*Percent*	*Percent*
1929	18	String	8,469	4.6	24.8	0.8
	18	Tape	11,848	3.0	7.2	1.1
1930	24	String	8,504	1.1	25.1	1.0
	24	Tape	8,760	1.1	14.2	1.8
1931	12	String	4,132	3.2	25.0	.5
	12	Tape	4,984	3.1	13.6	1.8

CONTROL OF OVERGROWTHS

METHODS AND MATERIALS

In experiments for the control of the various overgrowths on the unions of piece-root-grafted nursery apple trees, four factors have been considered: (1) The kind of seedling employed, (2) the treatment of seedling roots with various antiseptics, (3) the manner of making the graft, and (4) the material used for wrapping the graft. As is pointed out later in this paper and in more detail by Hildebrand (*11*) and Riker and Hildebrand (*29*), wounds produced by soil insects represent another factor deserving special consideration.

The practice of excision of overgrowths and subsequent antiseptic treatment is not considered in the present paper, because of previous unpromising results.

A considerable range of conditions was sought in making the different trials. For this reason tests were conducted in representative nurseries in a number of States, namely, Iowa, Kansas, Minnesota, Missouri, Nebraska, Oklahoma, and Wisconsin. The complexity of the problem and the multiplicity of factors involved have hindered the rapid progress of the work and must be considered in the interpretation of the results secured. Various factors have operated alone or in combination in different nurseries during the same season and in the same nursery in different seasons. Certain control measures successful in one place have failed completely in another. Consequently, corresponding variability has been introduced into repetitions of the trials, the more important trials having been repeated in several places in the same season and in the same place in different seasons. The trial grafts differed from the corresponding control grafts in only one particular.

The varieties of trees employed differed in different localities. In general, varieties grown in comparatively large numbers and those reported to be susceptible to enlargements at the union were chosen for the tests, but some of the less popular and less susceptible varieties were used also.

The age of the trees used differed in different localities. In the Northern States the trees stood 3 or 4 years in the nursery; in the Southern States they were lifted after 1 or 2 growing seasons. In a number of cases trees grown only 1 season were examined in order to secure a preliminary idea of the efficiency of the methods being employed. The examination was made by removing the soil from around the tree until the union could be seen. When this was done, special care was taken not to injure the trees, and after the examination the soil was replaced. Because of the labor involved, especially during wet and cold weather, only a small number of trees were examined, usually 100 receiving the same treatment and 100 controls; in some cases only 50 of each. These examinations were made at random in the larger plantings.

Most of the records of the different trials were kept in considerable detail. The presence or absence of an overgrowth on the underground parts of each tree was noted. Records were kept of the location, relative size, probable cause, and character of tissue of all the enlargements and of the presence, number, character, and size of hairy roots. However, in a number of cases only a summary of the condition at the union was recorded, especially when a representative distribution had been secured for a variety in a given locality and when the numbers involved were large.

Because of the great volume of the records only synopses of the data are represented here, except in one representative instance.

RELATION OF TREES TO CONTROL FACTORS

The age of the nursery apple trees has an important bearing upon the significance of the results secured. In accord with the results of Melhus and Maney (*16*), the percentage of graft knots on 1-year-old trees was found to be of doubtful value in predicting the percentage on the same trees in later years, but is important in showing the efficiency of control measures applied at grafting time. A comparison of the results with 1-year-old trees and the same trees when 2 years old shows that important factors continue to operate in the second season.

Comparisons between the percentages of smooth unions on first- and second-season trees, some of which had been wrapped with tape and others with string, are shown in table 2. The 10 trials involved records of data on 14,435 trees. The observations on first-season trees were made by direct examination after the soil had been removed from the unions. The soil was then replaced. Because of the labor involved, a comparatively small number of trees in each lot (between 50 and 100 taken at random through the entire planting) were examined.

TABLE 2.—*Percentages of smooth unions on grafts of various plantings of nursery apple trees at different ages*

State	Variety	Amount of graft knot	Smooth unions on trees wrapped with—							
			Tape				String			
			First season	Second season	Third season	Fourth seaon	First season	Second season	Third season	Fourth season
			Pct	*Pct.*	*Pct*	*Pct*	*Pct.*	*Pct*	*Pct.*	*Pct*
Kansas	Wealthy	Great	96	77			54	37		
	do	do	86	67			34	39		
Minnesota	do	do	100	94			46	48		
	Okabena	do	100	58			16	4		
Iowa	do	Small	95	99			55	74		
	Oldenburg	do	100	98			68	74		
	do	do	92	94			56	79		
	Yellow transparent	do	100	97			84	89		
	do	do	91	96			82	88		
	Wealthy	do	97	90			84	86		
Nebraska	do	do	96		81		70		81	
	do	do	100		94		92		82	
Minnesota	Bayfield	Great	84			49	48			18
	do	do	82			36	58			29
	Wealthy	do	92			59	40			14
	do	do	100			67	46			34
	Okabena	do	84			44	36			13
	do	do	100			42	16			8

When the amount of graft knot was large, several results were noted which deserve comment. Infectious hairy root was the predominant type of overgrowth. The trees wrapped with tape generally appeared to be protected during the first year against the several factors causing the development of graft knots, but this protection had little influence during the second year when the agencies producing graft knot continued to operate. The question as to just what these agencies were and the method by which they acted has been discussed in more detail by Riker and Hildebrand (*29*). These writers have also reported that the possibility of long incubation periods for bacteria on actively growing trees under favorable conditions seems too remote to have much bearing upon this question. Siegler and Piper (*40*) have reported that causal bacteria could be isolated rarely if at all from aerial crown-gall inoculations after 65 days.

When the amount of graft knot was relatively small it appeared that many of the unions wrapped with string which had shown wound overgrowths or excess callus by the end of the first year became smooth by the end of the second year. This process had been noticed earlier by Melhus and Maney (*16*), with respect to poor unions. The time required for excess callus to become smooth depended upon the size of the callus, the vigor of the tree, and the absence of other knot-producing agencies. No cases were noted of recovery from infectious types of overgrowths.

Comparisons between the percentages of smooth unions on trees of different ages, e.g., on first- and third-season trees and on first- and fourth-season trees, gave similar results. A total of 8,906 trees was examined in these trials.

These results have a definite bearing upon the interpretation of the various control studies. It appeared quite clear that the first season is not necessarily the most important for the development of graft knots; however, it may be the most important for interpreting the results of the control measures applied at grafting time. When the amount of graft knot was large and the graft knots were predominantly of the infectious hairy-root type, the first season was often less important than the seasons that followed. On the other hand, when the amount of graft knot was small and the graft knots were mostly of the wound-overgrowth type, there was often no material change from the first season. Except during an epidemic of hairy root, the percentages of smooth unions following string wrapping increased during the second season; however, they never reached the point where they exceeded the percentages of smooth unions following tape wrapping. No epidemics of crown gall were observed. Since control measures thus far have been applied almost entirely at grafting time and during the first season, these studies show that under certain conditions the methods used cannot be expected to yield fully satisfactory results. However, they can, perhaps, reduce the intensity of an epidemic by lowering the percentages of trees that provide inoculum for later years. Thus far there is no evidence to show that the crown-gall (*31*) and hairy-root (*11*) bacteria may gain entrance into the host tissue in any way except through wounds. Consequently, these studies point to the root-chewing insects in the soil and to injuries during cultivation as agents in the production of epidemics, certainly after the first season and probably during the first season.

INFLUENCE OF SEEDLING STOCK USED

To determine what influence the seedling stock might exert on the number of enlargements that develop from piece-root grafts, 32 trials were made involving 31,000 grafts. Seedlings from France and from the States of Colorado, Kansas, Vermont, and Washington were compared. The trials were made during several years, in Iowa, Kansas, Missouri, Nebraska, and Oklahoma, with scions of the following varieties: Rome Beauty (dark red sport), Delicious, Golden Delicious, McIntosh, and Wealthy. The details of these studies are omitted because of their volume.

In all but nine of the individual trials the percentage differences of smooth trees on grafts with different seedlings were relatively small. When the percentage differences were 10 or more the values are listed as examples to show the variations. In these trials the percentage differences in smooth trees obtained from the use of different lots of seedling stock were 18, 32, 12, 35, 32, 12, 10, 11, and 11. The first five of these examples showed a predominance of infectious hairy root. In these cases it seems clear that in each trial one lot of the seedlings employed carried the hairy-root organism on the surface to a much greater extent than the other. Out of the 64 lots of seedlings employed in the 32 trials, 39 lots yielded over 80 percent of smooth trees when they were lifted. The relatively large percentage of smooth trees and the diversity in type of the enlargements that were found suggest that hairy-root bacteria on the surface of seedling roots was not a very serious factor in a majority of the cases studied. Although different lots of seedlings showed considerable variability, no correlation in the amount of graft knot was found between one year and the next with the seedlings from one region or even from one nursery. But in several cases it was found that various shipments of seedlings from a particular nursery gave consistently unfavorable results in several different States in the same year. However, the next year the seedlings from that same nursery were among the best employed. To summarize these results, it appears that, in accord with the results of Waite and Siegler (*49*) and Siegler and Piper (*41*), circumstantial evidence was found indicating that the causal bacteria may be carried on the surface of certain lots of apple seedlings. It therefore seemed desirable to try isolations from the surfaces of suspected seedlings.

Out of 19 attempts at isolation, positive results were secured in 15; repetitions of these trials gave similar results. The hairy-root bacteria were obtained from seedling apple roots in 24 out of 26 trials. The identity of the bacteria was determined by certain cultural characters and by successful inoculations into plants.

Other factors besides the presence of hairy-root bacteria should be mentioned in considering the incidence of overgrowths in grafts made with different lots of seedlings. The physiology of the seedling in relation to ripeness and to resistance is well worthy of further study. Attention has been given by several writers (*8*, *11*, *34*, *42*) to the question of resistance of different varieties of fruit trees to crown gall and related diseases. It therefore seemed desirable to determine whether apple seed from different sources would produce seedlings that differed in resistance to graft knots.

The exact source of the seed was not easy to determine. However, in a few instances the seed was followed from the variety of apple that produced it, and in three cases the seed was traced to individual trees. Two experiments with seed from Hopa (crab) and Meader trees may be mentioned together. The special seed was planted in the same field with commercial seed, and the seedlings were used to make piece-root grafts with McIntosh scions. At the end of two growing seasons from the time the grafts were planted, data were recorded for 500 trees from each seed source. The trees grafted on seedlings grown from Hopa seed were 89 percent smooth; those from Meader seed also were 89 percent smooth. Those from four corresponding lots of commercial seed were, respectively, 58, 56, 56, and 61 percent smooth. In a similar trial involving similar numbers, seed from Wealthy trees was employed to grow seedlings on which were grafted Jonathan scions. The trees grafted on seedlings grown from Wealthy seed were 91 percent smooth. Those from three corresponding lots of commercial seed yielded, respectively, 74, 78, and 78 percent smooth trees. Although these results are too few to justify conclusions, they are sufficiently striking to show the desirability of further work in this direction. Such work presents the difficulty, however, of differentiating between the amount of true resistance and the amount of response to causal bacteria carried on the surface or within small injuries. Although these Hopa, Meader, and Wealthy seedlings received the same treatment as the commercial seedlings, the possibility remains that they may merely have escaped surface contamination by the hairy-root bacteria.

VALUE OF CERTAIN ANTISEPTICS

The presence of the hairy-root bacteria on the surfaces of certain lots of seedlings points to the possible value of antiseptic treatments for seedling roots.

Certain antiseptics appear to have promise at times in preventing infection when the grafts are made from seedlings carrying undesirable bacteria. Since Waite's work in 1909,[4] antiseptics have been employed with varying success by a number of workers (*7, 10, 12, 15, 16, 20, 31, 36, 44, 48, 49*, and *50*). The present writers made trials with a considerable number of antiseptics in the hope of controlling the various overgrowths due to bacteria that gain entrance into the plant at grafting time. However, since it has been determined, as explained earlier, (1) that not all graft knots are due to bacteria, (2) that not all seedlings carry infectious bacteria in significant numbers, and (3) that a considerable percentage of the graft knots are caused by hairy-root bacteria that gain entrance to the tissue months after the union is established (*11, 29*), it is not surprising that the results of work with antiseptics have failed to be uniformly promising.

A considerable number of substances were given laboratory, greenhouse, and field trials. The best of these were selected for more extensive trials. Thirty-five treatments, with corresponding controls, were made in the preparation of approximately 25,000 grafts in Iowa, Kansas, Minnesota, Missouri, Oklahoma, and Wisconsin with scions of the following varieties: Rome Beauty (dark red sport), Jonathan, Stayman Winesap, Wealthy, and Yellow Transparent. Since most of the antiseptics were not found to be satisfactory, mention is made of only three.

Hydroxymercurichlorophenol sulphate (Semesan) was used in 15 trials, which are summarized in table 3.

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[4] It appears that Waite was the first to employ an antiseptic (formaldehyde) solution as a dip for apple stocks and scions before grafting. Although this early work was unpublished it has already been noted (*49*).

Table 3.—*Effect of three antiseptics on occurrence of malformations at the unions of piece-root-grafted nursery apple trees*

Antiseptic	Trials	Age of trees	Wrapper	Total trees examined	Stand	Trees showing indicated condition at union		
						Smooth	Small knot[a]	Large knot[b]
	Number	*Years*		*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Semesan	10	1-3	String	2,172	52	74	8	18
Controls	10	1-3	do	2,666	52	70	10	20
Semesan	5	1-2	Tape	1,675	66	85	1	14
Controls	5	1-2	do	2,550	63	81	1	18
Silver nitrate	6	1-3	String	2,150	67	86	4	10
Controls	6	1-3	do	2,676	53	82	6	12
Silver nitrate	8	1-3	Tape	4,098	54	95	2	3
Controls	8	1-3	do	4,204	66	94	1	5
Mercuric chloride	2	2	do	834	64	94	1	5
Controls	2	2	do	1,792	64	80	0	20

[a] This class included all enlargements, regardless of cause, smaller in cross measurements than half the diameter of the tree

[b] This class included all enlargements larger in cross measurements than half the diameter of the tree

In 10 of the trials the grafts were wrapped with string or cloth and treated in a 1:400 solution according to the directions of Waite and Siegler (*49*). In the 5 other trials only the seedlings were treated and the grafts were wrapped with adhesive tape. The control grafts received no treatment whatever. In one trial in Minnesota, after 1 season of growth with a string wrapper, the treated grafts showed 68 percent smooth, whereas the untreated grafts showed 42 percent smooth. In another trial, in Missouri, after 2 seasons of growth with a cloth wrapper, the treated grafts showed 56 percent smooth and the untreated grafts 88 percent smooth. The Minnesota trial showed a difference of 26 percent in favor of the treatment; whereas the Missouri trial showed a difference of 32 percent against the treatment—great and contradictory differences. However, in most cases the differences were rather small and more of them were in favor of the treatment than against it. The averages given in table 3 show several percent in favor of the treatment. The average differences are considered to be within the range of experimental error. The stand appeared not to be influenced by the treatment. These results appear in general accord with the following statement by Siegler (*39*): "We experimented with the use of Semesan which gave good results in earlier years but which recently has not proved so efficacious." As explained earlier, the factors that operate to induce overgrowths in one place may be very different from those in another. The case in which 32 percent more enlargements were induced in the treated grafts than in the controls was very puzzling. An explanation was sought in a laboratory study of this substance.

The efficiency of the hydroxymercurichlorophenol sulphate was determined against the crown-gall organism with a modified Rideal-Walker technic as used by Keitt, Shaw, and Riker (*14*). In the absence of any organic matter a dilution of 1 in 800 at 20° C. killed a measured concentration of a 48-hour-old washed culture of the bacteria in 10 minutes, but not in 5 minutes. As was to be expected, the presence of a little soil extract or other organic material considerably reduced the efficiency of this antiseptic. It seems likely that, under nursery conditions, enough soil might easily be present to counteract the germicidal effect of the chemical. Under such conditions it appears probable that the treatment might spread rather than destroy any bacteria present.

Silver nitrate, 1:1,000, gave rather poor results in the control of overgrowths. The seedling roots were dipped in this substance and held for 2 minutes; no subsequent treatment was made. In 6 trials the grafts were wrapped with string and in 8 with tape. A summary of these trials is included in table 3. The differences between the percentages of smooth trees resulting from the treated grafts and those resulting from the untreated grafts somewhat favor the treatment but are still within experimental error. However, the injury from the silver nitrate produced a noticeable reduction in stand.

The statistical method of Fisher (*5*) was applied to these data, in consultation with Dr. R. A. Brink, of the University of Wisconsin, in order to see whether the treatments might be more effective than they appeared. The significance of the mean difference in relation to the standard deviation of the difference was calculated. For treatments with hydroxymercurichlorophenol sulphate and with silver nitrate, the values of P were 0.7 and 0.6, respectively. These calculations, which are omitted because of their large volume, corroborate the conclusion derived from inspection that these treatments had little if any effectiveness in general practice.

Mercuric chloride, 1:1,000, in limited trials has given more promising results than any other antiseptic employed. The average of two trials shown in table 3 indicates that if used in conjunction with tape wrappers it may be effective, especially if the seedlings carry infectious bacteria. Other trials now under way appear thus far to corroborate these results.

Tested by the laboratory method described above, mercuric chloride showed considerably greater toxicity than Semesan. A dilution of 1 in 15,000 at 20° C. killed a measured concentration of a 48-hour-old culture of crown-gall bacteria in 10 minutes, but not in 5 minutes. Further work with antiseptics seems desirable.

COMPARISON OF WELL-MATCHED TONGUE AND WEDGE GRAFTS

The type of graft in relation to the prevention of various overgrowths at the union of piece-root apple grafts was examined in a number of trials. Hedgcock (*10*) reported that poorly made grafts were more likely than well-made ones to produce callus enlargements and were also likely to become infected. In addition, Riker and Keitt (*31*) showed that poorly made grafts united only a part of the scion to the root and that a situation similar to a partial girdle resulted. Siegler (*38*) suggested that the fit of the grafts has been overemphasized. Melhus et al. (*17*) found that better unions might be secured with wedge grafts. A number of different series of well-fitted tongue and wedge grafts were made. Various lots of these grafts were wrapped with adhesive tape; others were wrapped with string. The results of these tests are presented in condensed form in table 4.

These studies involved 72 different trials, with a total of 27,981 tongue grafts and 21,759 wedge grafts. The results were obtained in 1928 to 1931, inclusive. The experiments were made in Iowa, Kansas, Minnesota, Missouri, Oklahoma, and Wisconsin on the following varieties: Bayfield, Rome Beauty (dark red sport), Delicious, Dudley, Early Harvest, Golden Delicious, Jonathan, Maiden Blush, Okabena, Oldenburg, Red Wine, Wealthy, Whitney (crab), and Yellow Transparent. The examination of first-year trees, as already explained, was made by removing the soil from around the unions of trees taken at random and not by lifting the trees. The data on comparative lots of 1-year-old trees were taken under one set of conditions, while those on 2-, 3-, and 4-year-old trees were taken under different conditions. Consequently, comparisons are possible between trees of the same age, but not between trees of different ages.

Table 4.—*Effect of tongue and wedge grafts on occurrence of malformations at the unions of piece-root-grafted nursery apple trees*

Wrapper	Trials (Number)	Graft	Age of trees	Total trees examined	Stand	Trees showing indicated condition at the union		
						Smooth	Small knot[a]	Large knot[b]
			Years	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
Tape	14	Tongue	1	1,893	69	96	3	1
	14	Wedge	1	1,485	58	93	4	3
	13	Tongue	2	7,716	53	92	1	7
	13	Wedge	2	6,265	65	92	1	7
	7	Tongue	3	3,505	71	89	0	11
	7	Wedge	3	3,072	55	89	0	11
	3	Tongue	4	630		48	4	48
	3	Wedge	4	464		51	5	44
	Summary 37	Tongue	1-4	13,744	65	89	2	9
	37	Wedge	1-4	11,286	60	89	2	9
String	10	Tongue	1	926	65	59	17	24
	10	Wedge	1	850	57	62	18	20
	14	Tongue	2	8,459	75	74	4	22
	14	Wedge	2	6,681	63	75	3	22
	8	Tongue	3	4,249	71	73	1	26
	8	Wedge	3	2,449	69	72	1	27
	3	Tongue	4	603		24	4	72
	3	Wedge	4	493		15	5	80
	Summary 35	Tongue	1-4	14,237	70	65	7	28
	35	Wedge	1-4	10,473	61	65	7	28
Tape and string	Summary 72	Tongue	1-4	27,981	68	78	4	18
Do	72	Wedge	1-4	21,759	60	78	4	18

[a] This class included all enlargements, regardless of cause, smaller in cross measurements than half the diameter of the tree

[b] This class included all enlargements larger in cross measurements than half the diameter of the tree.

Considerable variations in the percentage of overgrowths at the unions were found in both directions in the comparison of tongue and wedge grafts. These variations occurred whether the grafts were wrapped with string or tape. It appeared from these trials that there was no significant difference between tongue and wedge grafts in the reduction of overgrowths at the union. These results are not necessarily opposed to those of Melhus et al. (*17*), for apparently these writers were concerned primarily with callus developments, whereas the present writers considered all the various kinds of overgrowths discussed earlier.

The reduced average stand of the wedge grafts wrapped with string as compared with that of tongue grafts seemed to have little significance because of the great variations in the results and because these variations favored first one and then the other type of graft. Fisher's method (*5*) was applied to the data on stands of tongue and wedge grafts at the end of the first season, to determine the significance of the mean difference in relation to the standard deviation of the difference. The value of *P* was 0.4, indicating that the difference had little if any significance. However, it should be noted that wedge grafts wrapped with string were more likely than tongue grafts to come apart before they were planted. Likewise, the wedge grafts had a greater tendency to send up sprouts from the seedling roots. When the grafts were wrapped with tape, the possibility of their coming apart and of the roots sending up sprouts was reduced.

EFFECT OF KIND OF WRAPPING USED

For reducing the percentage of enlargements at the unions of piece-root-grafted nursery apple trees, the kind of wrapping used was the most important single factor studied. A considerable number of different kinds of wrapping were tried, including string, waxed string, chemically treated string, string covered with wax after wrapping, paper, raffia, chemically treated raffia, raffia covered with wax, cloth, waxed cloth, and a number of kinds of adhesive tape. Hedgcock (*10*) found that, of the wrappers he tested, cloth was the best. In the present trials cloth was found to be better than any of the others except adhesive tape. Consequently detailed reports of the long series of trials in which wrappers other than adhesive tape were employed have been omitted. However, certain determinations were made which deserve mention. It was found that by means of various treatments with different chemicals the string wrappers could be preserved for practically any length of time during the growing season. It was also observed that under average conditions a string wrapper that remained longer than 12 weeks was likely to produce girdling. The time necessary for decay of the wrapper varied considerably under different environmental conditions. When the ground was unusually moist the trees grew more rapidly and the wrapper decayed more quickly. Conversely, when the soil was comparatively dry the wrapper lasted longer, but the trees did not grow so rapidly. The strength of the string decreased rather gradually as the season progressed. This diminution apparently depended on the ready access of soil organisms, for any protected portion of the string retained its strength much longer than the unprotected portions.

The adhesive tape chiefly employed was essentially a cloth wrapping to which had been added a plaster mass. The adhesive-tape wrapper provided several valuable features, including (1) reasonable cost, (2) easy application, (3) firm wrapping, which prevented injury to the union during subsequent manipulations and which facilitated handling, (4) a waterproof covering, which prevented the entrance of undesirable material, (5) a mechanical prevention against the development of excess callus on the surface of the union, and (6) a barrier for some months against insect injury at the union.

Not all kinds of adhesive tape were satisfactory. A small number of trials with electrician's friction tape and with tape spread with "surgeon's mass" gave such unpromising results that they were discontinued. Certain other special tapes in which various chemicals were incorporated sometimes showed no advantage over ordinary tape. Some of these special tapes had serious disadvantages; a few of them considerably reduced the stands, and two samples that were tried prevented any of the grafts from growing. Several of these special tapes were comparatively slow to decay in the soil and for that reason might last long enough under some conditions to produce girdling. The adhesive tape[5] employed in these trials was rather similar to that used extensively by the medical profession, but with modifications in the interests of economy.

The manner of application of the adhesive tape was found to be important both in relation to speed and to the results secured. Scions 5 to 6 inches long and roots 3 to 4 inches long were used for the grafts, which were well made from good materials. The method of applying the adhesive tape was equally successful with either tongue or wedge grafts. A roll of ½-inch-wide adhesive tape was mounted on a roller at the side of the operator. The graft was turned in the hand and the tape was applied in a spiral wrap over every part of the union and overlapped the edge slightly. Only enough tape was used to make a water-tight covering over every part of the cut surface. The tape was so applied that no more than two thicknesses of material circled the graft at any one point. After a little practice it was possible to wrap 400 to 600 grafts in an hour. The amount of tape used varied with the size of the grafts, but on an average approximately 110 yards of tape one half inch wide wrapped 1,000 grafts.

The tests with wrappers covered a wide range of conditions. Results are reported for the years from 1925 to 1931, inclusive. The unions of the first-year trees taken at random were examined by removing the soil to a suitable depth for inspection. Sometimes only 50 tape-wrapped and 50 string-wrapped trees were examined, but at other times there were 100, 250, or 300 in each lot.

The results from the use of tape wrappers as compared with those from the use of commercial wrappers have been summarized for each trial (table 5) and have been grouped according to the age of the tree at the time the data were taken. As explained earlier, no comparison should be made between the data on trees of different ages, for the trials are not comparable.

Table 5 presents the results of 145 trials with adhesive tape wrappers with a corresponding set of controls. The trials with tape involved 55,326 trees, and the controls involved 55,105. This table shows no significant difference in stand because of the different wrappers. It shows variability in the amount of graft knot according to age of tree, variety, and the State in which the trees were grown. It also indicates a distinct increase in the percentage of smooth trees correlated with the use of adhesive tape. At the same time it emphasizes the variation between individual trials and indicates that different factors operate in the same nursery in different seasons and in different nurseries in the same season.

A summary of table 5 is given in table 6.

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[5] Manufactured under the name "nurseryman's tape."

TABLE 5.—*Effect of string*[a] *and adhesive-tape wrappers on occurrence of malformations at the unions of piece-root-grafted apple trees of various ages*

State	Variety	Unions showing indicated condition on trees wrapped with—							
		String				Tape			
		Total examined	Smooth	Small knot[b]	Large knot[c]	Total examined	Smooth	Small knot[b]	Large knot[c]
		Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
	FIRST-YEAR TREES								
Iowa	Oldenburg	50	68	16	16	50	100	0	0
	do	50	56	34	10	50	92	8	0
	Wealthy	100	55	14	31	100	95	3	2
	do	100	84	10	6	100	100	0	0
	do	100	69	18	13	100	99	1	0
	do	100	84	2	14	100	97	2	1
	Yellow Transparent	50	84	8	8	50	100	0	0
	do	50	82	12	6	50	96	2	2
Kansas	Wealthy	50	54	26	20	50	96	2	2
	do	50	34	30	36	50	86	10	4
	do	50	36	16	48	50	86	10	4
	Yellow Transparent	300	71	29	0	300	78	22	0
	do	300	68	32	0	300	71	29	0
Minnesota	Bayfield	50	48	20	32	50	84	6	10
	do	50	58	16	26	50	82	10	8
	Okabena	50	36	26	38	50	84	6	10
	do	50	16	30	54	50	100	0	0
	Wealthy	50	40	20	40	50	90	4	6
	do	50	40	20	40	50	92	4	4
	do	50	46	30	24	50	86	12	2
	do	50	46	30	24	50	100	0	0
	do	50	42	18	40	50	100	0	0
	do	50	42	18	40	50	98	2	0
	do	50	52	14	34	50	94	6	0
	do	100	83	3	14	100	99	0	1
Nebraska	do	100	70	13	17	100	100	0	0
	do	100	70	13	17	100	96	3	1
	do	100	98	1	1	100	98	2	0
	do	100	92	4	4	100	100	0	0
Oklahoma	do	250	7	24	69	250	29	27	44
Wisconsin	do	250	53	27	20	250	91	2	7
	SECOND-YEAR TREES								
Iowa	Ben Davis	155	72	1	27	215	94	0	6
	Delicious	50	94	2	4	50	100	0	0
	do	50	82	6	12	50	100	0	0
	do	50	84	4	12	50	96	2	2
	do	50	94	4	2	50	96	2	2
	do	50	96	4	0	20	100	0	0
	do	50	86	0	14	20	100	0	0
	do	50	98	0	2	50	96	0	4
	do	50	94	2	4	50	96	0	4
	Oldenburg	992	74	3	23	1 000	98	0	2
	do	399	79	2	19	1 014	94	0	6
	Dudley	50	88	8	4	50	88	0	12
	do	50	94	2	4	50	94	2	4
	Early Harvest	1 005	84	1	15	940	98	0	2
	Fameuse	568	79	2	19	1 009	97	0	3
	Florence (crab)	414	97	0	3	445	99	0	1
	Gano	573	80	0	20	695	97	0	3
	Golden Winesap	768	78	1	21	555	99	0	1
	McIntosh	1 012	84	2	14	1 020	94	1	5
	Red Siberian (crab)	512	96	0	4	575	98	0	2
	Wealthy	935	74	5	21	1 016	99	0	1
	do	50	70	16	14	50	96	2	2
	do	50	78	2	20	50	94	4	2
	do	1 000	93	0	7	1 000	97	0	3
	do	1 000	93	0	7	1 000	98	0	2
	do	1 000	90	0	10	1 000	97	0	3
	do	1 000	90	0	10	500	99	0	1
	do	279	86	0	14	1 330	90	0	10
	Whitney (crab)	1 034	91	0	9	1 049	99	0	1
	Yellow Transparent	259	88	2	10	285	96	1	3
	do	1 180	89	2	9	1 093	97	0	3

[a] Including string, waxed string, and waxed raffia.

[b] This class includes all enlargements, regardless of cause, smaller in cross measurements than half the diameter of the tree.

[c] This class includes all enlargements not classified as small.

TABLE 5.—*Effect of string and adhesive-tape wrappers on occurrence of malformations at the unions of piece-root-grafted apple trees of various ages*—Continued

SECOND-YEAR TREES—Continued

State	Variety	Unions showing indicated condition on trees wrapped with—							
		String				Tape			
		Total examined	Smooth	Small knot	Large knot	Total examined	Smooth	Small knot	Large knot
		Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
Kansas	Ben Davis	654	49	4	47	649	60	5	35
	Jonathan	612	74	1	25	579	88	0	12
	do	612	74	1	25	595	91	1	8
	do	588	70	1	29	612	87	1	12
	McIntosh	567	89	1	10	431	95	0	5
	do	559	89	1	10	518	95	0	5
	do	1,005	58	3	39	512	76	2	22
	do	534	56	2	42	515	73	1	26
	do	550	56	4	40	551	80	1	19
	do	112	61	3	36	534	83	0	17
	Wealthy	898	37	7	56	810	77	4	19
	do	771	39	4	57	743	67	4	29
	do	775	35	6	59	743	67	1	29
	do	456	52	3	45	462	71	0	29
	Yellow Transparent	496	45	5	50	502	52	2	46
Minnesota	Okabena	50	4	4	92	50	58	10	32
	Wealthy	50	48	8	44	50	94	0	6
	do	250	62	14	24	250	96	2	2
Missouri	Ben Davis	305	79	1	20	273	92	0	8
	Rome Beauty (dark red sport)	1,000	68	14	18	1,000	89	9	2
	do	1,000	68	14	18	800	78	20	2
	Oldenburg	1,496	85	1	14	731	95	0	5
	Early Harvest	707	93	1	6	137	98	0	2
	Fameuse	1,080	89	1	10	996	97	0	3
	Maiden Blush	648	90	1	9	836	99	0	1
	Wealthy	178	57	2	41	216	80	2	18
	do	243	57	2	41	316	82	3	15
	do	243	57	2	41	342	84	3	13
	do	500	47	1	52	45	87	0	13
	do	500	47	1	52	68	88	2	10
	do	1,000	94	0	6	1,000	98	0	2
	do	1,000	81	1	18	1,000	89	1	10
	do	1,000	46	2	52	1,000	94	1	5
	do	250	74	3	23	250	90	1	9
	Winesap	227	90	1	9	320	97	0	3
	Yellow Transparent	693	47	2	51	583	87	0	13
Nebraska	Wealthy	50	82	6	12	50	92	6	2
	do	50	82	6	12	50	100	0	0
	do	250	66	21	13	250	89	4	7
Oklahoma	Delicious	447	84	0	16	238	98	0	2
	do	438	72	1	27	310	87	2	11
	do	473	94	1	5	465	97	0	3
	Jonathan	436	94	0	6	636	98	0	2
	York Imperial	367	66	1	33	441	89	1	10
Wisconsin	Wealthy	250	72	12	16	250	90	8	2
	do	250	69	22	9	250	95	3	2
	do	250	86	8	6	250	96	2	2
THIRD-YEAR TREES									
Iowa	Dudley	174	90	0	10	34	91	0	9
	do	36	94	0	6	92	90	0	10
	Wealthy	101	80	0	20	104	94	0	6
	do	270	85	1	14	182	96	0	4
Minnesota	do	50	64	0	36	50	94	0	6
Nebraska	do	134	66	5	29	120	85	5	10
	do	306	65	11	24	215	91	0	9
	do	600	78	2	20	600	88	1	11
	do	600	78	2	20	600	88	0	12
	do	600	81	0	19	600	93	0	7
	do	600	81	0	19	600	94	0	6
	do	600	80	0	20	600	80	0	20
	do	600	80	0	20	600	89	0	11
	do	376	81	0	19	375	81	0	19
	do	361	82	1	17	382	94	0	6
	Whitney (crab)	533	85	1	14	529	93	0	7
	do	645	86	1	13	742	95	1	4
	Yellow Transparent	961	79	1	20	972	87	1	12
	do	813	84	0	16	765	87	0	13

TABLE 5.—*Effect of string and adhesive-tape wrappers on occurrence of malformations at the unions of piece-root-grafted apple trees of various ages*—Continued

THIRD-YEAR TREES—Continued

State	Variety	Unions showing indicated condition on trees wrapped with—							
		String				Tape			
		Total examined	Smooth	Small knot	Large knot	Total examined	Smooth	Small knot	Large knot
		Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
Wisconsin	Bayfield	589	50	3	47	509	67	1	32
	Oldenburg	717	46	1	53	113	65	2	33
	Goodnue	195	60	0	40	542	68	0	32
	Northwestern Greening	402	71	5	21	736	71	2	27
	Perkins	763	66	0	34	310	74	1	25
	Red Wing	631	33	0	67	240	63	0	37
	do	471	66	5	29	482	82	1	14
	Wealthy	300	88	4	8	300	96	2	2
	do	1,243	48	0	52	424	73	0	27

FOURTH-YEAR TREES

State	Variety	String: Total examined	String: Smooth	String: Small knot	String: Large knot	Tape: Total examined	Tape: Smooth	Tape: Small knot	Tape: Large knot
Minnesota	Bayfield	161	18	9	73	117	49	6	45
	do	184	29	8	63	265	36	7	57
	Okabena	111	8	1	91	57	12	2	86
	do	60	13	4	83	87	44	3	53
	Wealthy	308	34	5	61	362	67	2	31
	do	308	34	5	61	308	67	4	29
	do	272	14	2	84	331	59	3	38
	do	272	14	2	84	260	59	6	35

TABLE 6.—*Summary of table 5 on experiments showing total or average effect of string*[a] *and adhesive-tape wrappers on occurrence of malformations at the unions of piece-root-grafted nursery apple trees of various ages*

Trials (number)	Wrapper	Age of trees	Total trees examined	Stand	Trees showing indicated condition at the union		
					Smooth	Small knot[b]	Large knot[c]
		Years	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
31	String	1	2,900	57	57	19	24
31	Tape	1	2,900	59	91	6	3
78	String	2	39,555	60	75	3	22
78	Tape	2	38,521	63	91	1	8
28	String	3	13,974	63	73	2	25
28	Tape	3	12,118	59	85	1	14
8	String	4	1,676		21	4	75
8	Tape	4	1,787		53	4	43
Summary:							
145	String	1–4	58,105	59	68	6	26
145	Tape	1–4	55,326	60	88	2	10

[a] Including string, waxed string, and raffia and wax.

[b] This class included all enlargements, regardless of cause, smaller in cross measurements than half the diameter of the tree.

[c] This class included all enlargements larger in cross measurements than half the diameter of the tree.

The superiority of adhesive-tape wrapping for reducing the percentage of enlargements on piece-root-grafted apple trees in most cases is beyond question. In none of the 145 trials in which there was more than 10 percent of disease were the string wrappers found to yield a higher percentage of smooth trees than the tape wrappers.

This alone places the mathematical probability that the tape is valuable in very significant figures.

The degree of superiority of the tape is difficult to estimate because of the variability of the results. In 20 trials there was less than 10 percent of disease development on the checks. Consequently, in such trials, control measures had little chance to show their value. On the other hand, some extreme differences occurred, such as differences of 40, 42, 48, and 56 percent in favor of the adhesive tape. The detailed results show differences ranging from 0 to 58 percent. The significance of the mean difference in relation to the standard deviation of the difference when at least 10 percent of knots were present was calculated, according to the method of Fisher (*5*), for trees of each age. When the value of P was less than 0.05, the mean difference in relation to the standard deviation of the difference was considered significant. The values of P were as follows: For the first-year trees, 0.02; for the second-year trees, 0.09; for the third-year trees, 0.12; and for the fourth-year trees, 0.02. In considering these values the variable factors already mentioned should be held in mind, for they make questionable the application of such mathematical treatment to some large portions of this data. It is obvious that certain influences which might affect the older trees, as explained earlier, did not affect the first-year trees. All the results with the fourth-year trees were taken in the same northern nursery during the same year. Consequently, the different trials were subject to fewer variables; this doubtless accounts for the low value of P. With a sufficiently large number of repetitions in several places and in successive years, the writers think that greater variations would probably be found. If similarly isolated groups of trials among the second- and third-year trees are taken, correspondingly low values of P are found.

Satisfactory control with adhesive tape was secured in the great majority of cases but not always. Examples may be found in table 5 in which the percentage of smooth trees from tape-wrapped grafts, while better than the controls, was only 29, 36, 42, 44, 49, 52, 58, 59, 60, etc. It appears from studies described by Riker and Hildebrand (*29*) and by Siegler and Piper (*41*) that results like these may be explained in two ways. (1) Infection of the union at the time of grafting may take place before the application of the adhesive-tape wrapper. This is perhaps pertinent when the discrepancy appears as it did once in Oklahoma on first-year trees. Such results resemble those secured from inoculations made at grafting time. (2) Infection may occur through injuries made by cultivation or by soil insects after the wrapper has decayed. This possibility has been already considered in relation to second-, third-, and fourth-year trees. This was doubtless the manner of infection on the first-year Yellow Transparent trees grown in Kansas, as recorded in table 5. Fortunately, detailed seasonal development records are available on these trees (*29*). Except for the one case in Oklahoma, these trees represent the worst failures on 1-year-old trees that the writers have experienced with tape wrappers.

The importance of infection at grafting time has been given consideration. Considerable emphasis has recently been placed on this one factor by Siegler and Piper (*41*). In an effort to discover the effect of adhesive tape upon infected unions, a number of grafts were inoculated with the hairy-root organism and then some were wrapped with string and the others with tape. At the end of the first season, both the string-wrapped and the tape-wrapped lots showed 100 percent of hairy root. Repetitions of these trials the next year gave, respectively, 95 and 100 percent hairy root. These experiments showed that ordinary adhesive tape has little if any effect on bacteria present in the union at the time of grafting. In view of this evidence it appears that in many cases reported in tables 2 and 5 infection at the union at grafting time had comparatively little direct influence on the results. This is shown by the high percentage of smooth tape-wrapped trees at the end of the first season, suggesting that control measures based only on the importance of infection at grafting time may lead to disappointment in many cases. However, when considered from the standpoint of providing a source of inoculum for spread in the nursery by soil fauna during the latter part of the first season and during later growing seasons, as indicated by Riker and Hildebrand (*29*), it assumes more importance.

Difficulty with the adhesive tape has been encountered in three ways, none of which was serious. (1) The mechanical operation of wrapping the grafts and of cutting or tearing the tape at the proper place caused difficulty only at first. (2) Girdling of the trees during the first summer was found in about 10 percent of one planting of grafts. In this case too much tape had been wrapped about the unions, and the grafts were planted in very sandy soil. Dry weather prevailed for some time during the early part of the growing season, with the result that the tape wrapper was not sufficiently moist to decay. Even under these conditions no girdling was found on the trees that had received the right amount of wrapper. (3) A slight roughening of the bark beneath the plaster mass was noted in a few cases with certain varieties. Necrotic areas were observed which penetrated into the cortex for a short distance. Roughness of the union has sometimes been confused with the discolored residual particles of the plaster mass.

DISCUSSION

In the control of graft knots the seed used for growing seedling apple trees has been found to be of considerable importance. Since different varieties of apple trees have been shown to differ widely in their susceptibility to hairy root, it seemed probable that a similar difference in susceptibility might be found in seedlings grown from the seed of these varieties. A limited amount of evidence presented in this paper shows promise in this line of investigation. But aside from their relative resistance, which may be influenced not only by genetic constitution but also by conditions attending growth and harvesting, the seedlings may supply one or more of the other important factors.

The seedlings used for grafting may carry pathogenic bacteria. How these bacteria arrive at the surface of the seedlings is not yet perfectly understood. They may be in the soil where the seedlings are grown. However, a seemingly more important factor appears after the seedlings are dug. Ordinarily they are collected in bundles, placed in a heap, covered with packing (frequently old packing that might be classed as refuse), watered, and allowed to stand in order to "sweat off" the leaves. During this procedure it is obvious that knot-producing bacteria on a very small percentage of infected seedlings or in the packing material might spread widely over the seedlings. There appear to be two possible remedies for this situation: (1) To prevent the spread of the bacteria; and (2) to destroy the bacteria by chemical treatments.

The bacteria carried on the surface of seedlings are aided in their entry into the union by certain cultural practices. In their effort to prevent desiccation of the seedling roots in the grafting room some nurserymen keep the roots wrapped in moist material until the minute of making the cut. Under such circumstances it has been observed repeatedly that the soil from the surface of the root may be carried over the cut surface by the knife, not only introducing any bacteria present into the union but also placing a layer of soil particles between parts of the scion and root and thus making union more difficult. Consequently, seedling roots that are clean and dry seem preferable during grafting to those that are wet and covered with soil. This appears to be quite important when the seedlings carry hairy-root bacteria, and deserves further experimental study.

The use of antiseptics on seedlings that carry hairy-root bacteria appears desirable. The determination of whether or not the infectious bacteria are carried on the surface of seedling roots is comparatively easy. By means of the technic developed by Patel (*21*) and Riker et al. (*27*), any well-equipped bacteriological laboratory might make the determinations. However, by no means all the apple seedlings carry infectious bacteria in sufficient numbers to be of primary consequence. Here again there is variation in different seedling nurseries during the same year and in the same nursery in different years. While some of the trials reported in tables 2 and 5 show that the bacteria were carried on the seedling and entered the union at grafting time, a larger number show that the union was invaded later on.

Wedge grafts appear to have no advantage over tongue grafts in the amount of graft knot developing. There was practically no difference when they were wrapped with tape. When the grafts were wrapped with string or raffia the wedge grafts had a somewhat greater tendency than tongue grafts either to come apart before planting or to send up sprouts from the root. On an average, the tongue grafts showed a slight advantage in stand.

The use of the adhesive-tape wrapper has been perhaps the most important single factor in the prevention of graft knots. Its function is rather complex. In the first place, inoculations at the union when the grafts were made indicated that the tape had little if any effect upon the entrance of bacteria at that time or on the development of infection by the bacteria that gained entrance. However, it had several other functions: (1) It prevented the further entrance of soil, water, and bacteria; (2) it reduced greatly any chance of injury to the union during various manipulations; (3) it encouraged better union between scion and root; (4) it prevented superficial development of excess callus; (5) some nurserymen have reported that it reduced the growth of mold at the union; and (6) it prevented root-chewing soil fauna from reaching the union for a number of months.

Root-chewing soil insects, including white grubs (Phyllophaga), wireworms (Elateridae), and fungus gnats (Mycetophilidae) (*29*), may play an important role in the development of infectious hairy root not only during the first season but also during succeeding seasons. Obviously, control measures applied only at the time the grafts were made could have little effect on this factor except as they might reduce the amount of readily available inoculum. According to common entomological observation, the comparatively mild winters during the last few years probably enabled a greater number of insects to survive and have correspondingly increased their importance as a factor in the graft-knot problem. While abundant moisture in the soil favored the growth of the trees it also favored the production of callus, the activity of the insects near the surface, and the chances of entrance into the plants of any bacteria present in the soil. This serves in part to explain why graft knots are so much worse in moist than in dry years. In view of this situation, which deserves further study, such land as pasture that has been favorable to root-chewing insects seems relatively unsuited for apple grafts.

The following practices promise to contribute to the reduction of graft knots at the unions of apple trees grown from piece-root grafts:

(1) Use of apple seed from relatively resistant trees as soon as it is available.

(2) Treatment with an antiseptic of seedling roots suspected of carrying knot-producing bacteria.

(3) Use of clean dry roots when grafting.

(4) Use of a suitable wrapper, such as the adhesive tape described.

(5) Planting in soil which has been so handled that it is relatively free from root-chewing insects such as white grubs, wireworms, and fungus gnats.

SUMMARY

Graft knots of the types discussed in this paper cause severe losses in apple nursery stock propagated by piece-root grafting.

Nursery trees having infectious hairy root made, on an average, slightly less growth than smooth trees. Trees remained alive and grew slightly when all the roots but those in the hairy-root overgrowth had been removed.

The problem of controlling the overgrowths is complex, because there are several different kinds of enlargements, arising from different causes. The different kinds of overgrowths occur in various sorts and degrees of mixture.

The various overgrowths appear well distributed in the nursery. In the case of infectious hairy root, a certain amount of spread in the nursery row from hairy-root trees has been indicated.

A large percentage of the overgrowths occur at the unions. This percentage has been reduced on an average in the last 5 years, doubtless owing to improved nursery practice.

Examinations of a number of plantings have shown correlations between the length of time the trees stayed in the nursery row and the percentage of overgrowths. Although in some cases the initiation of most of the overgrowths could be traced to grafting time, in many others it was traced to the second or later seasons.

In some instances the surfaces of seedlings were found to carry hairy-root bacteria which were sources of inoculum at grafting time. In other instances this factor seemed of relatively little importance. The growing of seedling apple trees from the seed of relatively resist-

ant apple varieties gives promise of being a factor of some importance in the control of graft knots.

Antiseptic treatments of seedlings carrying bacteria that cause overgrowths seem to promise some measure of control.

Well-matched tongue grafts produced as many smooth unions as wedge grafts. Tongue grafts appeared to have certain minor advantages over wedge grafts.

Adhesive-tape wrapping appeared to be better than any other wrapping employed and to be the most important single factor among control measures. However, tape wrappers did not prevent infection at the time the grafts were made.

Control measures are discussed in relation to one another and to common nursery practice.

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TECHNIC FOR OBTAINING SPERMATOZOA FOR PHYSIOLOGICAL DAIRY STUDIES AND ARTIFICIAL INSEMINATION [1]

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INTRODUCTION

Semen may be collected from the vagina of the recently bred cow with the hand, by aspiration, or with a sponge. Semen from the vagina is satisfactory for determining whether the bull has ejaculated normal active spermatozoa during the mating, but it is unsatisfactory for use in physiological studies of spermatozoa because it is mixed with the secretions of the cow. Furthermore, collecting semen from the vagina for artificial breeding is wasteful.

A method of obtaining semen from the bull by massage of the accessory genital organs has been developed in the Bureau of Dairy Industry's physiological laboratory at Beltsville, Md. It has not been determined, however, what effect the continuous practice of obtaining semen in this way would have on the health and usefulness of the bull.

REVIEW OF THE LITERATURE

Komarov and Nagaev [2] designed a special rubber bag which they placed in the vagina of the cow. By careful technic in conducting the mating they were successful in obtaining a superior quality of semen as compared to that collected directly from the vagina with a sponge. Later, according to a report by Walton,[3] these workers used an artificial vagina and a "dummy" animal, which they claimed worked satisfactorily.

Case [4] in 1925 reported that "we procure the semen either by pressing on the seminal vesicles through the rectum, or from the vagina of the recently bred cow." In a letter to the authors in November 1932 Case described his method of massaging the seminal vesicles to obtain semen and also stated that he had used the method successfully 10 years ago in collecting semen for artificial impregnation.

ANATOMY OF THE BULL'S ACCESSORY GENITAL ORGANS

At Beltsville it was found that the seminal vesicles of the bull do not contain spermatozoa, but that the spermatozoa are in the ampullae of the ductus deferens.

The seminal vesicles and ampullae are easily identified and are so located that it is possible to manipulate the one without disturbing the other. It is more difficult to locate and manipulate the prostate of the bull because it consists of two parts and is protected by heavy muscle. The body of the prostate consists of a band which stretches across the neck of the urinary bladder and the origin of the urethra.

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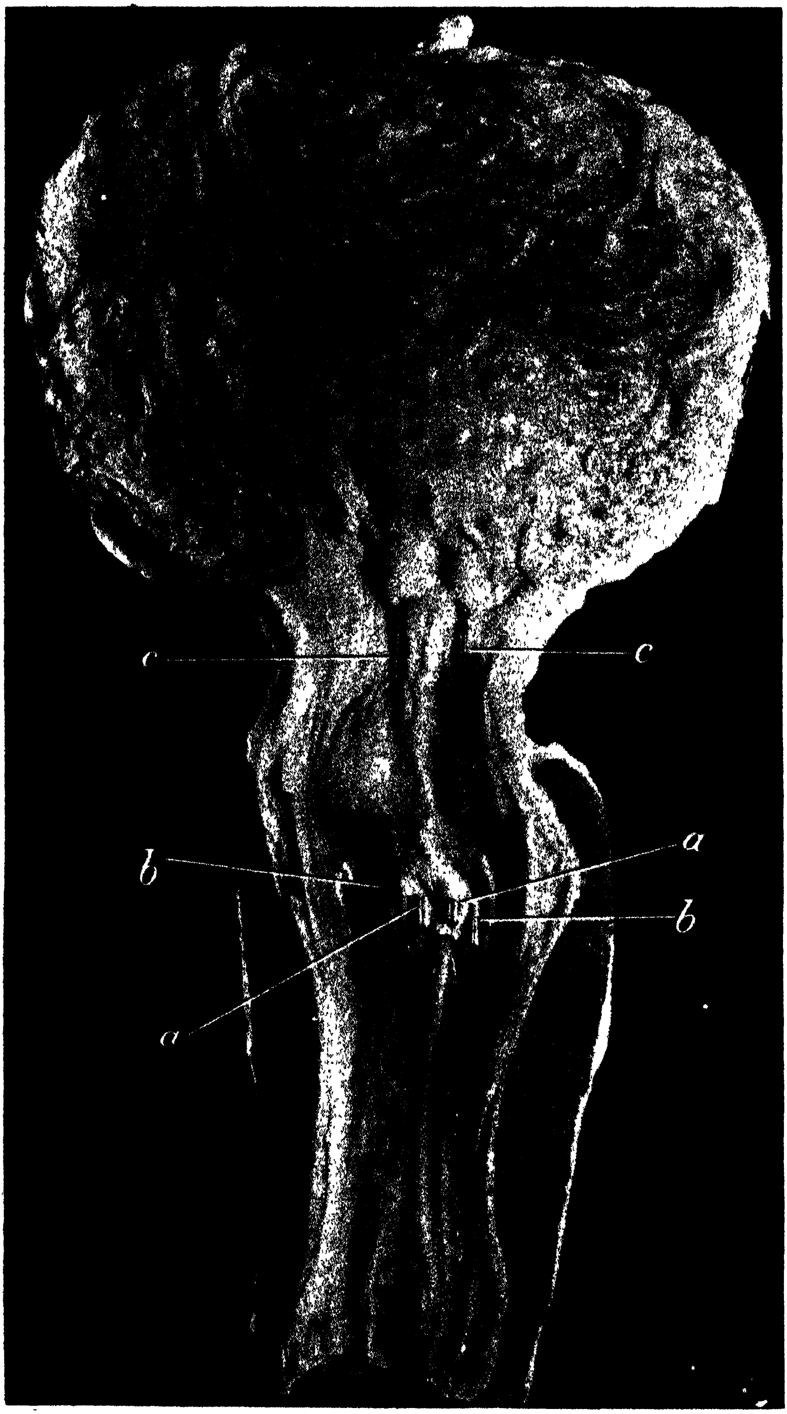

FIGURE 1.—Pelvic urethra and urinary bladder slit ventrally and laid open: *a*, Openings of ductus deferens; *b*, openings of seminal vesicles; *c*, uretral orifices.

It is about an inch and a half transversely and about half an inch in width and thickness. The pars disseminata surrounds the pelvic part of the urethra and is concealed by the urethral muscle (figs. 2 and 3).

As shown in figure 1 the ducts from the seminal vesicles and ductus deferens do not enter the urethra in a common opening; the ampullae of the ductus deferens have large lumen and the seminal vesicles have small tubules. The relation of these organs to each other is shown in figure 2.

METHOD AND RESULTS OF ITS USE

With a hand in the bull's rectum, from 7 to 10 inches, the seminal vesicles were massaged with backward strokes, and a turbid fluid flowed from the prepuce. It contained only epithelial cells in the majority of cases. In like manner the ampullae of the ductus deferens were massaged and a turbid fluid flowed out which contained only spermatozoa in the majority of cases. In some instances the ampullae were massaged first, but better results were obtained when the seminal vesicles were massaged first, because, while the ampullae were being massaged, the seminal vesicles released some of their fluid and both spermatozoa and epithelial cells were obtained. When the seminal vesicles were massaged first the epithelial cells came out, leaving only spermatozoa in the fluid obtained from the ampullae. Usually about 2 minutes of massaging gave excellent results.

In figure 3 the genital organs are shown replaced in the right half of a bull carcass. The weight of the urinary bladder has pulled the genital organs forward about an inch from the normal position in the live bull. The hand is shown massaging the seminal vesicles in figure 3, *A*, and the ampullae in figure 3, *B*.

Figure 4 shows the method of collecting the fluids as they flow out and figure 5 the comparative density of the fluids from the seminal vesicles and from the ampullae. Microscopical views of cells found in the two fluids are shown in figure 6.

Eighteen bulls ranging from 2½ to 12 years in age were used in the first 100 trials to obtain semen by massage. Epithelial cells or debris were found in 100 samples of fluid from the seminal vesicles; and in 6 of these spermatozoa were found, always from bulls that had not been used for long periods. In these six cases it is probable that the ampullae were disturbed while the seminal vesicles were being massaged.

Of the 100 trials in massaging the ampullae 81 were successful and spermatozoa were obtained from 15 bulls. Thirty-one trials were made on one bull and each time enormous quantities of spermatozoa were obtained. No spermatozoa were obtained from the ampullae of three bulls. Two of these were tried only once and the other one twice. They were disposed of before further trials were made. Failure to obtain spermatozoa was experienced in 19 trials. It was assumed that the ampullae had been emptied just previous to the time of massaging. This was indicated by the volume and tone of the ampullae, the small flaccid tube yielding no spermatozoa and the large firm tube yielding many spermatozoa.

The quantity of fluid collected from the seminal vesicles at one time varied from 0.5 to 21 cc and that from the ampullae varied from 0.5 to 23 cc.

CONCLUSIONS

Massaging the accessory genital organs of the bull is a practical way of obtaining semen for physiological studies. For artificial breed-

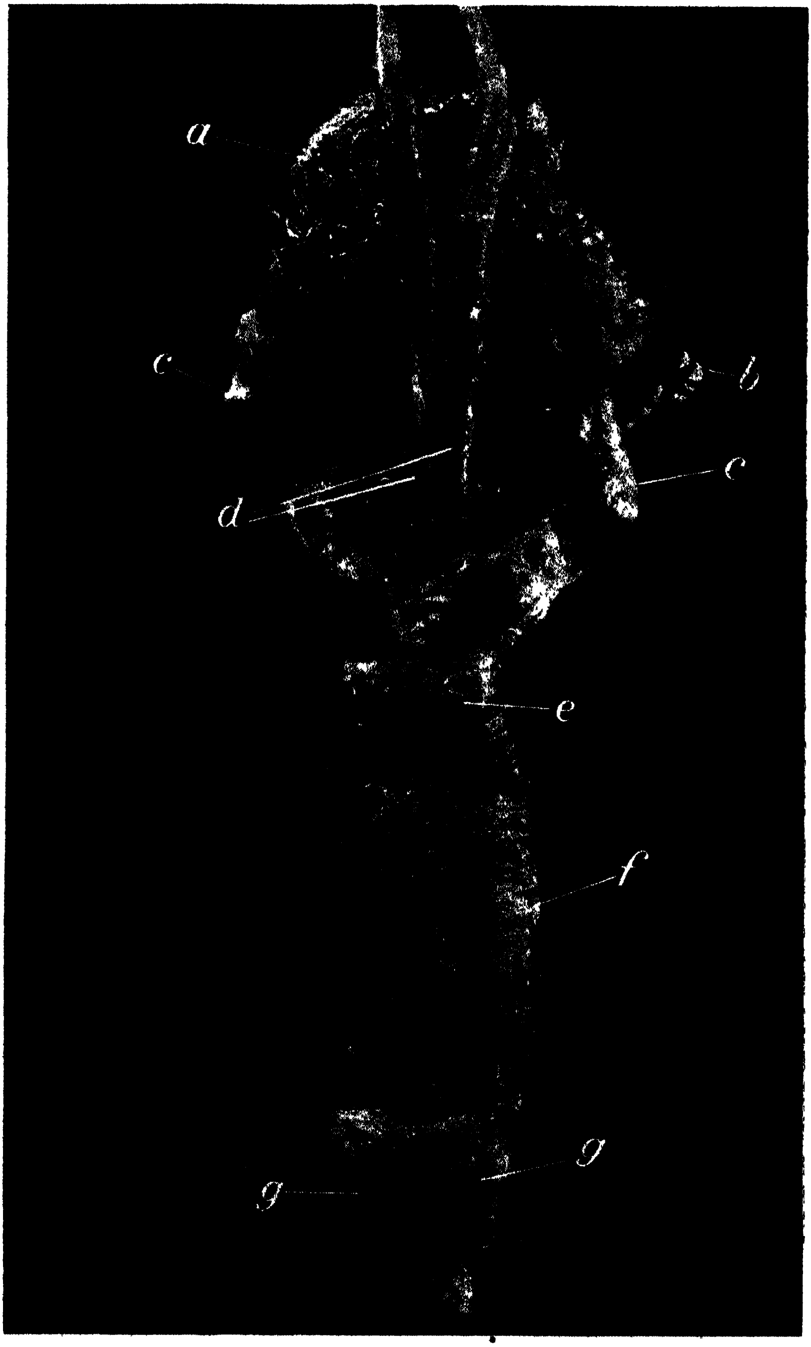

FIGURE 2.—Dorsal view of the internal organs of a bull: *a*, Bladder; *b*, ureter; *c*, seminal vesicles; *d*, ampullae; *e*, body of prostate; *f*, pelvic urethra; *g*, bulbo-urethral (Cowper's) glands.

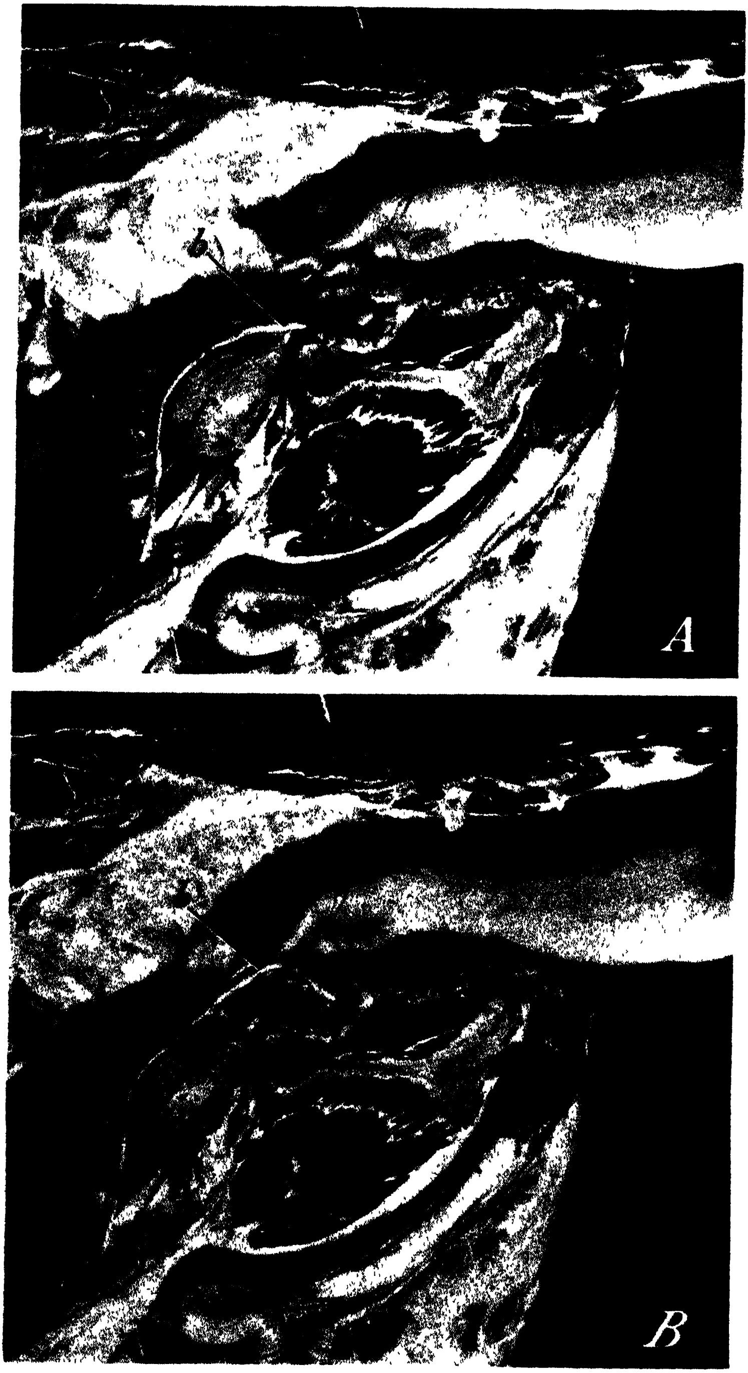

FIGURE 3.—Position of the genital organs of the bull and method of manipulating them: *A*, Massaging the seminal vesicles; *B*, massaging the ampullae of the ductus deferens. The organs are: *a*, Seminal vesicles; *b*, ampullae; *c*, body of prostate; *d*, pelvic urethra; *e*, bulbo-urethral (Cowper's) glands; *f*, urinary bladder; *g*, pubis

FIGURE 4.—Method of collecting semen with funnel and test tube.

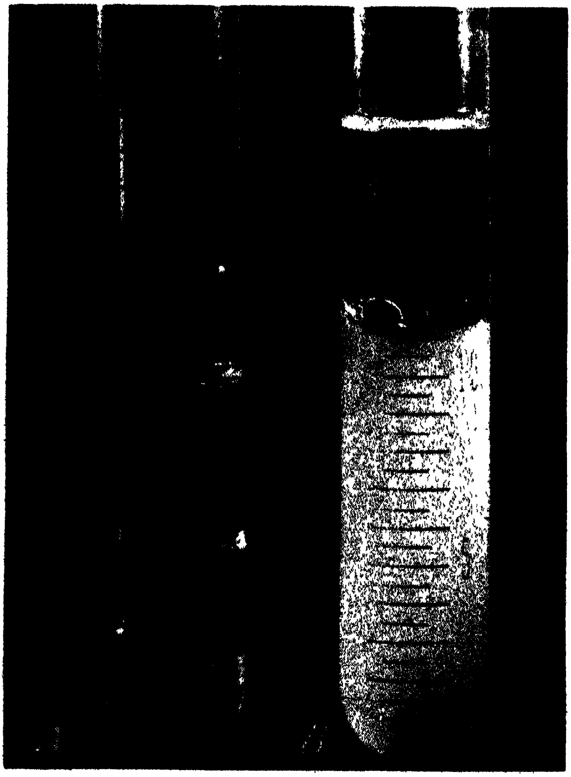

FIGURE 5.—Material collected from (*A*) seminal vesicles and (*B*) ampullae.

ing purposes it is desirable to massage the ampullae, for from this organ will be obtained the greatest volume of semen containing active spermatozoa. The method is also useful with valuable breeding bulls

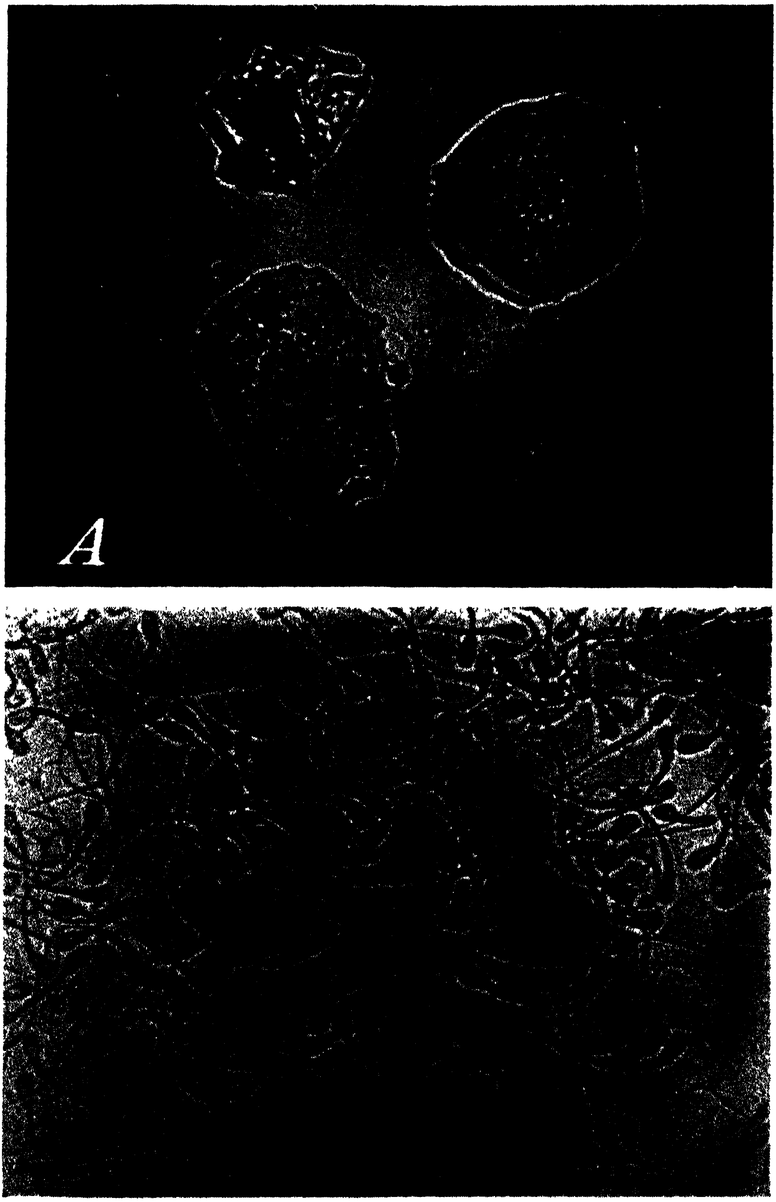

FIGURE 6.—*A*, Epithelial cells from seminal vesicles; *B*, spermatozoa from ampullae. × 450.

that are unable to serve cows in the normal manner because of injury. Other advantages of collecting semen directly from the bull for use in artificial breeding are that it prevents waste of semen and produces semen free from extraneous matter.

APHELENCHOIDES XYLOPHILUS, N. SP., A NEMATODE ASSOCIATED WITH BLUE-STAIN AND OTHER FUNGI IN TIMBER[1]

By G. STEINER, *senior nematologist, in charge*, and EDNA M. BUHRER, *junior nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

A new case of unusual ecological conditions to which nematodes have adapted themselves has been found in a nematode species apparently specialized to live in timber affected by blue-stain and other wood fungi. This new species is described herein.

ECOLOGICAL RELATIONS

The first observation of *Aphelenchoides xylophilus*, n.sp., the timber nema, dates back to 1929, when a small piece of wood that had been cut, in the process of roofing, from the top of a green pole of longleaf Louisiana pine (*Pinus palustris* Mill.) was received from Orange, Tex.[2] This piece of wood had streaks of a bluish color caused by blue-stain fungi. The nemas were found in these streaks and in bordering portions. Larval specimens, males and females, were observed. Although they were not numerous, a dozen or more specimens could be found in a small portion of wood when soaked and dissected properly. Soaking the wood in water activated the nematodes, whereas drying the wood induced dormancy. Some tests showed revival of the nematodes after a dormancy of 1 year but not after 2 years.

Later, through the courtesy of Ross W. Davidson, of the Division of Forest Pathology, Bureau of Plant Industry, there were received four different plate cultures of wood fungi in which nematodes had developed. All of these nematodes proved to be *Aphelenchoides xylophilus*. Three of the cultures were from a sawmill in Bogalusa, La., and were also obtained from blue-stained logs of *Pinus palustris*. These logs had previously been attacked by beetles of the genus *Ips*, which, according to Davidson, usually carry the blue-stain fungus *Ceratostomella ips* Rumbold, but which in these three cases contained a brown fungus belonging probably to the genus *Trichosporium*, of the "Fungi Imperfecti."

The fourth culture on which the same species of nematode developed was obtained from a pine tree (*Pinus echinata* Mill.) that had been recently killed by an attack of the beetle, *Dendroctonus frontalis* Zimm., near Fairfax, Va. In this case the nematodes originated in the interior of unstained wood, one-sixteenth to one-fourth of an inch below the insect galleries. The fungus here associated with this nematode is said by Davidson to be entirely hyaline and also to belong

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[1] Received for publication Apr. 4, 1934; issued July 1934.

[2] Received through the courtesy of T. E. Snyder, of the Bureau of Entomology, U.S. Department of Agriculture, who received the wood from C. H. Lyon, chemist of the Texas Creosoting Co Mr. Lyon wrote: "All such poles came from an area including western Louisiana and southeastern Texas. The climate is hot and, at the time of finding that specimen, was very humid. The average annual humidity is given by the Government observer of this region as of 83 3 percent. The specimen had not been treated nor come in contact with creosote. It has been at all times exposed to weather."

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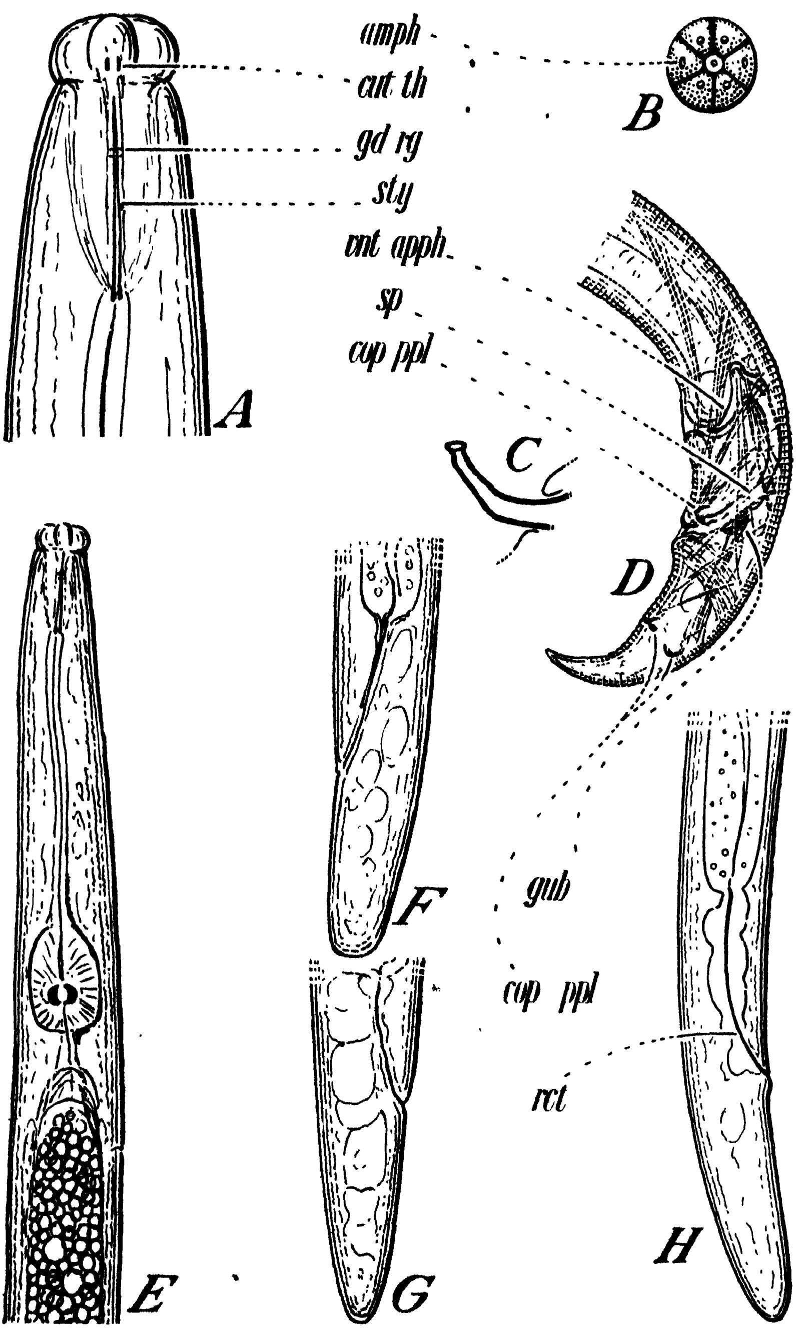

IGURE 1.—*Aphelenchoides xylophilus*, n.sp. *A*.—Head of female: *cut th*, Cuticular thickening in cephalic portion of alimentary tract; *gd rg*, guiding rings of stylet; *sty*, stylet. × 2,800. *B*.—Front view of head. *amph*, Amphid. × 1,370. *C*.—Extruded spicula showing circular expansion. × 1,370. *D*.—Tail of male: *vnt apph*, Ventral apophysis; *sp*, spicula; *cop ppl*, copulatory papillae (three pairs); *gub*; gubernaculum. × 1,060. *E*.—Anterior end of larva. × 1,060. *F* and *G*.—Tails of larvae, showing variation in shape. × 1,060. *H*—Tail of female: *rct*, Rectum. × 1,060

to the "Fungi Imperfecti." However, according to the same authority, this particular "isolation" was taken only a short distance (1 inch or less) from wood which was blue-stained by *Ceratostomella pini* Münch, a fungus associated with *D. frontalis*. It is thought that the nematode might also have been present in this blue-stained wood.

This constant association suggests that the nematode described herein uses the insects as carriers and probably feeds on the various fungi involved in the same association. Similar carrier relationships between nematodes and insects are known, especially in connection with bark beetles, dung beetles, flies frequenting fermenting substances, etc. In this respect the observations made herein offer nothing new, but the apparent specialization of this nematode to a life in wood and its association with fungi of the blue-stain type merit special attention.

TECHNICAL DESCRIPTION

Aphelenchoides xylophilus, n.sp

Like the other members of the genus, *Aphelenchoides xylophilus* is of slender shape and has the following dimensions:

$$♀\quad \frac{1.8}{1.0}\quad \frac{7.9}{1.5}\quad \frac{8.7}{1.5}\quad \frac{74.}{1\ 6}\quad \frac{96\ 2}{1.0}\qquad 0.9\text{ mm.}$$

$$♂\quad \frac{2\ 2}{1.2}\quad \frac{9.3}{1.6}\quad \frac{10.}{1\ 6}\quad \frac{M}{2.0}\quad \frac{95\ 9}{1\ 5}\qquad 0.77\text{ mm.}$$

The cuticle is very finely annulated (8 annules to 6μ in the head region); the head well set off; the tail of the larva and female more or less obtuse and slightly longer than the rectum (fig 1, *F*, *G*, *H*), that of the male conically pointed, ventrally curved, and slightly longer than the spiculum (fig. 1, *D*). A front view of the head shows a six-radiate cuticular structure. The lobes between the radii carry the sense organs in the order typical for the genus, i.e., on the lateral lobe the amphids, on each submedial lobe one papilla (fig. 1, *B*). The stylet is very fine and about one and one-half times as long as the head is wide; its knobs are minute; two fine guiding rings are present and the wall of the cephalic portion of the alimentary tract is reinforced by short cuticular thickenings (fig. 1, *A*). It seems that the esophageal glands open in the middle bulb of the esophagus in the manner typical of the genus; the bodies of the glands, however, have a dorsal situation outside the alimentary tract at the beginning of the intestine; they extend to about 85μ behind the esophageal bulb and have a strictly serial arrangement. The length of the rectum is about twice the anal body diameter. An obscure excretory pore opens ventrad of the nerve ring (fig. 1, *E*). The vulva is a narrow transverse slit but stands out rather well because the body narrows suddenly behind it The testis is outstretched forward to the right of the intestine, and ends about 300μ behind the esophageal bulb. The spicula resemble those of other members of the genus but have in addition an extremely well-developed ventral apophysis at the proximal end. In some specimens this apophysis seemed to connect with the ventral body wall (fig. 1, *D*), but in others no such connection was seen. Figure 1, *C*, shows the distal end of the spiculum as forming a circular expansion. A small gubernaculum is present. The copulatory musculature is shown in figure 1, *D*. There are two pairs of large, somewhat mammillate ventrosubmedial copulatory papillae (fig. 1, *D*), one pair at about the middle of the tail and the other just in front of the anus. A third pair seems to have a dorsolaterad position also in the middle of the tail.

DIAGNOSIS.—*Aphelenchoides* with an obtusely rounded conical tail in the larva and in the female, but pointed in the male, with a fine, barely knobbed buccal stylet. The spiculum of the male proximally with long ventral apophysis; a short, lineate gubernaculum present; male tail with large mammillate copulatory papillae; a pair ventrosubmedial in front of anus, a second pair ventrosubmedial in the middle of the tail, and a third dorsolaterad also in the middle of the tail. Associated with blue-stain and similar wood fungi.

TYPE HOST.—*Pinus palustris.*

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RELATION OF BARBERRY TO THE ORIGIN AND PERSISTENCE OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS[1]

By E. C. STAKMAN, *head of the Section of Plant Pathology, Minnesota Agricultural Experiment Station,* and *agent, Division of Barberry Eradication;* M. N. LEVINE, *pathologist, Division of Cereal Crops and Diseases;* RALPH U. COTTER, *associate pathologist,* and LEE HINES, *agent, Division of Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

It is now well known (*4, 5, 6, 9, 13*)[2] that new physiologic forms of *Puccinia graminis* Pers. may arise as the result of hybridization between existing forms or varieties on barberry. The evidence at present available indicates that hybridization probably accounts principally for the origin of forms, although Stakman, Levine, and Cotter (*9*) have shown that mutation in parasitism may also occur, though probably rarely. There is little information, however, regarding the origin of new forms through hybridization on barberries in nature. For this reason the writers made studies during the past several years to ascertain whether new physiologic forms could be obtained from aecial material and from uredial material near barberries. Preliminary statements of the results have been published (*7, 12*). These and other data have been combined with the results of earlier surveys and are presented in the following pages. Data are given also on the results of "selfing" pycnia resulting from inoculations with telial collections of unknown identity.

The writers were particularly interested in the varieties of *Puccinia graminis* that attack the common small grains; hence inoculations were made on barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), or on wheat (*Triticum vulgare* Vill.),[3] oats, and rye (*Secale cereale* L.), to obtain preliminary indications of the identity of the variety of rust in question. Barley is susceptible to both the *tritici* and the *secalis* varieties; hence, it sometimes was used in the preliminary inoculations to "screen out" the *poae* and *agrostidis* varieties, neither of which develops well on it. The determination of physiologic forms within rust varieties was made by revised keys originally developed by Stakman and Levine (*8*) for physiologic forms of *P. graminis tritici* Eriks. and Henn.; by Bailey (*1*) for physiologic forms of *P. graminis avenae* Eriks. and Henn., and by Cotter and Levine (*3*) for physiologic forms of *P. graminis secalis* Eriks. and Henn.

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[1] Received for publication Feb. 2, 1934, issued July, 1934 Cooperative investigation of the Divisions of Barberry Eradication and Cereal Crops and Diseases, Bureau of Plant Industry, U S. Department of Agriculture, and the Minnesota Agricultural Experiment Station. Since this paper was written, the Division of Barberry Eradication has been combined with the Divisions of Blister Rust Control, Citrus Canker Eradication, and Phony Peach Eradication in a single division designated the Division of Plant Disease Eradication. The new division has been made a part of the Bureau of Entomology and Plant Quarantine. Published as paper no. 1105 of the Journal series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 968.

[3] According to the rules of botanical nomenclature the name of this species is *Triticum aestivum,* but as *T vulgare* is in general use among agronomists and cereal pathologists and geneticists, the writers give preference to that form

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The relatively low percentage of infection resulting from inoculating with aeciospores may be due to several causes. *Puccinia graminis poae* Eriks. and Henn. and *P. graminis agrostidis* Eriks. and Henn.[4] are very prevalent on barberries in certain regions, and many of the collections probably were of these varieties. Then, too, most of the material was sent through the mails and much of it was not in good condition when received. Further, the germination of aeciospores is somewhat more uncertain and considerably more capricious than that of urediospores; and aeciospores do not usually retain their viability more than 3 weeks, even when kept cool and fairly dry.

VARIETIES ISOLATED

VARIETIES FROM AECIAL COLLECTIONS

Of 675 aecial collections of *Puccinia graminis* tested during the past 13 years, only 281 caused infection on wheat, oats, rye, or barley (table 1). It seems likely, therefore, that many of the collections were of the *poae* or the *agrostidis* variety. Of the 281 collections that caused infection on the common small grains, 96, or 34.2 percent, were of the *tritici* variety; 179, or 63.7 percent, were of the *secalis* variety; and only 6, or 2.1 percent, were of the *avenae* variety.

TABLE 1. *Isolation of Puccinia graminis varieties from rusted barberries in the field, 1920–32*

Year	Collections tested	Total cultures identified	Isolations of *Puccinia graminis* var.					
			Tritici		*Secalis*		*Avenae*	
	Number	*Number*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1920	8	4	3	75.0	1	25.0		
1921	14	6	5	83.3	1	16.7		
1922	9	3	1	33.3	2	66.7		
1923	6	2			2	100.0		
1924	12	6	1	16.7	5	83.3		
1925	9	5	3	60.0	2	40.0		
1926	19	12			11	91.7	1	8.3
1927	27	19	8	42.1	9	47.4	2	10.5
1928	93	60	23	38.3	35	58.4	2	3.3
1929	64	41	18	43.9	23	56.1		
1930	131	42	16	38.1	26	61.9		
1931	124	32	12	37.5	19	59.4	1	3.1
1932	159	49	6	12.2	43	87.8		
Total	675	281	96	34.2	179	63.7	6	2.1

It seems quite likely that the high percentage of collections of *Puccinia graminis secalis* is due to the fact (1) that quackgrass (*Agropyron repens* (L.) Beauv.) is susceptible to this variety, (2) that this grass is very prevalent in the Northern States, where barberries become rusted, and (3) that many of the remaining barberry bushes are along fence rows, in pastures, on the edges of wood lots, along streams, and in other uncultivated places, where quackgrass is likely to be abundant. Furthermore, *Hordeum jubatum* L. and other species of *Hordeum* are very susceptible to *P. graminis secalis*, as are also various species of *Elymus*. There is therefore opportunity for *P. graminis secalis* to develop abundantly. *P. graminis tritici* attacks not only wheat but also the same grasses that *P. graminis secalis* attacks, with the exception of *Agropyron repens*. As just explained, however, the great abundance and the advantageous habitat of the last-named grass give *P. graminis secalis* the advantage, despite the fact that the acreage of wheat attacked by *P. graminis tritici* is far greater than the acreage of rye attacked by *P. graminis secalis*. The relative paucity of collections of *P. graminis avenae* probably is due to the fact that *Dactylis glomerata* L., *Festuca* spp., *Alopecurus* spp., *Glyceria*, and the other grasses susceptible to this variety usually are not particularly abundant near barberry bushes (*11*).

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[4] The varietal name as written by Eriksson and Henning is *agrostis*. In order to conform with the other varietal names, however, the writers prefer to use the genitive form, *agrostidis*, as originally used by Eriksson.

VARIETIES FROM UREDIAL MATERIAL NEAR INFECTED BARBERRIES

In addition to determining the relative prevalence of the varieties of *Puccinia graminis* occurring in the aecial stage on barberry bushes in nature, the writers made a similar study of uredial material on grains and grasses in the immediate vicinity (within 100 yards) of infected bushes. This study includes only such rust as almost certainly resulted from infected bushes. The results are summarized in table 2.

TABLE 2.—*Isolations of Puccinia graminis varieties from uredial collections obtained in close proximity to infected barberries, 1919–32*

Year	Cultures identified	Isolations of *Puccinia graminis* var.					
		Tritici		*Secalis*		*Avenae*	
	Number	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1919	3	2	66.7	1	33.3		
1920	3	1	33.3	2	66.7		
1921	4	2	50.0	2	50.0		
1922	9	3	33.3	6	66.7		
1923	3			1	33.3	2	66.7
1924	4			3	75.0	1	25.0
1925	5	2	40.0	3	60.0		
1926	7	2	28.6	4	57.1	1	14.3
1927	21	9	42.9	8	38.1	4	19.0
1928	27	16	59.3	5	18.5	6	22.2
1929	7	4	57.1	3	42.9		
1930	8	5	62.5	1	12.5	2	25.0
1931	19	12	63.2	2	10.5	5	26.3
1932	18	14	77.8	4	22.2		
Total	138	72	52.2	45	32.6	21	15.2

A total of 138 collections of *Puccinia graminis* were identified. Of these, 72, or 52.2 percent, were of the *tritici* variety; 45, or 32.6 percent, of the *secalis* variety; and 21, or 15.2 percent, of the *avenae* variety. The fact that the percentage of collections of the *tritici* variety was higher than that of the *secalis* variety, whereas the opposite was true of the aecial collections, is probably due largely to conscious selection. The writers were most interested in determining physiologic forms of the *tritici* variety and therefore made special effort to collect grasses and grains likely to be infected with it. Hence the percentages do not necessarily give as accurate an indication of the relative prevalence of the varieties in nature as those for the aecial collections.

It is clear that a high percentage of *Puccinia graminis* on barberries in the Northern States is of the *secalis* variety. This variety of rust probably is more closely dependent on barberry for persistence from season to season than are the *tritici* and *avenae* varieties. This is partly because very little rye is grown in the South, where the uredial stage of *Puccinia graminis* can survive the winter. It is evident that there is appreciable development of stem rust of rye only in those regions where there are barberries. Stem rust of rye can be prevented, for practical purposes, by eradicating barberries. While it is perfectly clear that barberry eradication reduces the severity of rust attacks on wheat and oats also, investigations by the writers have shown that the uredial stage of these rusts often survives the winter in the far South and, under favorable conditions, urediospores may be blown northward, to cause infection on wheat and oats in the Northern States.

PHYSIOLOGIC FORMS ISOLATED

Attempt was made to identify the physiologic forms, both in aecial and uredial collections. In addition, barberries were inoculated in the greenhouse with collections of teliospores and the pycnia were "selfed", as described later. The primary object was to find out to what extent new physiologic forms were arising through hybridization in nature. It was desired also to ascertain to what extent barberries make possible the persistence of existing forms. Theoretically barberries should be of great importance in these respects, and, practically, the results show that they are.

PUCCINIA GRAMINIS TRITICI

Physiologic Forms from Aecial Collections

The identity of the physiologic forms represented in the 94 isolations of *Puccinia graminis tritici* was determined, 26 forms being identified. Some of them were of frequent occurrence and wide distribution, others rare and limited; some were quite virulent, others fairly innocuous (table 3 and fig. 1; see also table 5).

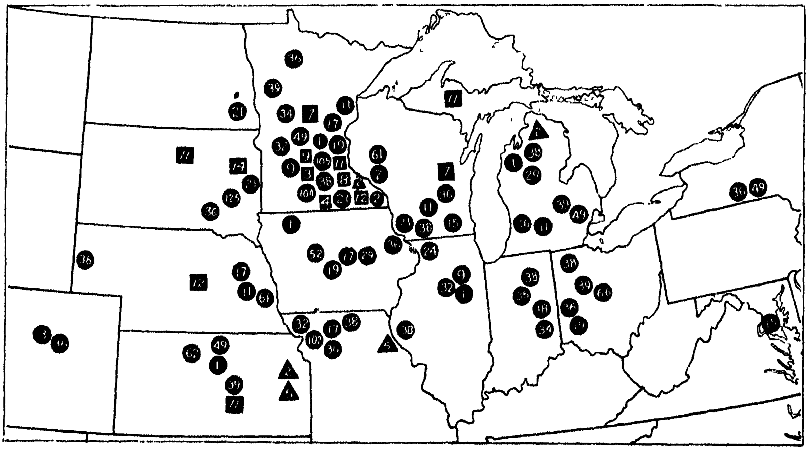

FIGURE 1.—Geographic distribution of physiologic forms (designated by number) of *Puccinia graminis*, isolated from naturally infected barberries. Circles indicate variety *tritici*, squares, *secalis*, triangles, *avenae*

It is worthy of note that so limited a number of isolations should have yielded so many different physiologic forms. This becomes especially striking when the number of physiologic forms isolated from field collections of infected grain plants is considered. In the latter case, 82 physiologic forms have been identified from more than 8,000 cultures made in the course of physiologic-form surveys; that is, on an average, not more than one form from each 100 uredial collections; whereas from aecial collections a different form was procured, on an average, from every fourth culture, although this ratio probably would not have persisted had more aecial collections been identified.

TABLE 3.—*Physiologic forms of Puccinia graminis tritici isolated from aecial collections, by States, 1929-32*

Form	Colorado	Illinois	Indiana	Iowa	Kansas	Maryland	Michigan	Minnesota	Missouri	Nebraska	New York	North Dakota	Ohio	South Dakota	Wisconsin	Total
	Number of times form was found in															
1		1		1	1		1	2								6
2								1							1	2
3	1															1
9		1						1								2
11							1	1		1					1	4
15						1									1	2
17				1				1	1	1						4
18			1													1
19				1				1					1			3
21								2				1		1	1	5
24		1														1
29				1			1									2
32		1						1	1							3
34			1					1								2
36	1		1	2			1	8	2	2	1		1	2	3	24
38		1	2				1	2	2				1		2	11
39					2		1	1					1			5
49					1		1	3			1					6
52				1												1
61										1					1	2
62					1											1
66													1			1
102									1							1
104								1								1
105								2								2
125														1		1
Total	2	5	5	7	5	1	7	28	7	5	2	1	5	4	10	94
Percent	2.1	5.3	5.3	7.5	5.3	1.1	7.5	29.8	7.5	5.3	2.1	1.1	5.3	4.2	10.6	100
Number of forms	2	5	4	6	4	1	7	15	5	4	2	1	5	3	7	26

Form 36 was most common and most widely distributed. It was isolated 24 times in a total of 94 identifications, and it was found in 11 of 15 States in which collections were made. Next in order of occurrence and distribution was form 38, with a total of 11 isolations from 7 States. This was followed by a considerable drop in both occurrence and distribution was form 38, with a total of 11 isolations from 7 States. This was followed by a considerable drop in both occurrence and distribution, with 6 isolations each of forms 1 and 49 from 5 and 4 States, respectively. Forms 3, 18, 24, 52, 62, 66, 102, 104, and 125 were isolated only once each from the aecial material collected.

Forms 62, 102, 104, and 105 never have been isolated from any source other than rusted barberry. The original collections of forms 61 and 66 were made from naturally infected barberries, although subsequently they were obtained also from rusted grain in the field.

These facts indicate strongly that in addition to spreading much inoculum early in the season barberry bushes are extremely important in at least two respects: (1) They enable many physiologic forms to persist from season to season and (2) they make possible the origin of new forms through hybridization. In 1919 it was pointed out by Stakman, Levine, and Leach (*10*) that the number of physiologic forms of *Puccinia graminis* seemed to be greater in those regions of the United States where there were many barberry bushes than in those where there were few. The results presented in this paper support this opinion and indicate clearly that barberry bushes should be eradicated in order to reduce the number of physiologic forms as well as to reduce the amount of inoculum, especially that of the early spring.

Of the forms isolated only from barberries and therefore probably produced on them during recent years, form 62 is the most virulent. It may cause heavy infection on all the differential hosts except Vernal and Khapli emmers. It is one of the most virulent of all forms, differing from form 15 only in its inability to infect Vernal normally and in its tendency to produce type x infection on Marquis, Reliance, and Kota wheats; on these wheats form 15 produces type 4 infection (table 5). Under favorable conditions type x may develop sufficiently to cause heavy rust attack; hence form 62 must be considered potentially dangerous. The other forms isolated only from barberries are not particularly virulent, although it is worthy of note that on Vernal emmer both form 104 and 105 produce type x infection, which is a heavier infection than that caused by most other forms.

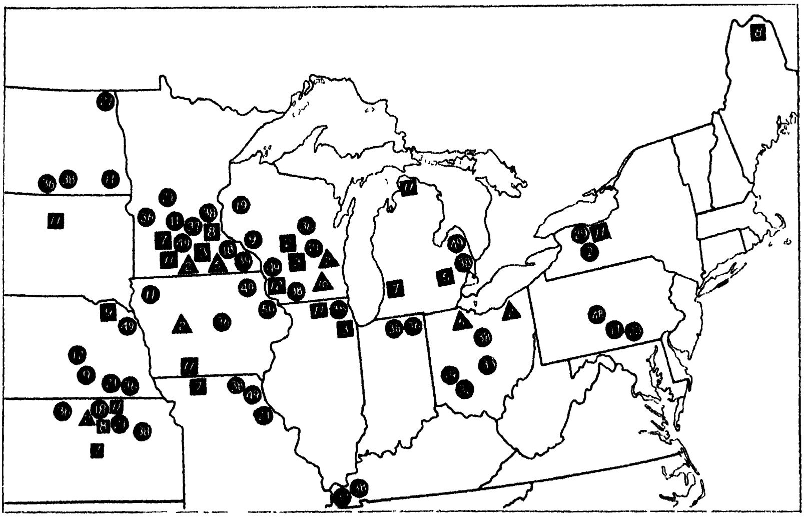

FIGURE 2.—Geographic distribution of physiologic forms (designated by number) of *Puccinia graminis*, collected in the vicinity of infected barberries. Circles indicate variety *tritici*; squares, *secalis*; triangles, *avenae*.

PHYSIOLOGIC FORMS FROM UREDIAL MATERIAL NEAR INFECTED BARBERRIES

The 71 collections of *Puccinia graminis tritici*, made on grains and grasses near infected barberries and completely identified, comprised 19 physiologic forms, a different form for approximately every four collections (table 4, fig. 2). These results support those obtained from inoculations with aeciospores. Here again it is clear that barberries are responsible for the persistence of many physiologic forms. When inoculations were made with urediospores collected at random, in regions where barberries rust and in those where they do not, a different form appeared in 1 collection in 100, on an average. But when inoculations were made with urediospores collected near rusted barberries, a different form was obtained in 1 culture of every 4.

TABLE 4.—*Physiologic forms of Puccinia graminis tritici isolated from uredial collections obtained near infected barberries, by States, 1919–32*

Form	Number of times form was found in—														
	Illinois	Indiana	Iowa	Kansas	Michigan	Minnesota	Missouri	Nebraska	New York	North Dakota	Ohio	Pennsylvania	Virginia	Wisconsin	Total
2									1						1
9								1						1	2
11			1			1				1					3
12								1							1
17												1			1
18				2		2									4
19											1			1	2
21				1		3	1	1		1				2	9
23												1			1
29									1						1
32											1				1
33											1		1		2
36	1	1	1	2		4		1		4				2	16
37						1									1
38		2		1	1	1	2			1	1		1	4	14
39						1									1
48												1			1
49			2		1	2	1	1						2	9
56			1												1
Total	1	3	5	6	2	15	4	5	2	7	4	3	2	12	71
Percent	1.4	4.2	7.1	8.5	2.8	21.1	5.6	7.1	2.8	9.9	5.6	4.2	2.8	16.9	100
Number of forms	1	2	4	4	2	8	3	5	2	4	4	3	2	6	19

Forms 36 and 38 were the most prevalent, constituting 22.54 percent and 19.72 percent, respectively, of the total collections identified. Form 38 has been very prevalent in the soft red winter wheat area of the United States, where there still are many barberries; it is also very abundant in northern Mexico. Forms 21 and 49 were next in order of prevalence. By consulting table 5 it will be seen that these four forms could cause heavy rust on the principal types of wheat commonly grown in the wheat-growing regions of the northern half of the United States. Marquis and similar varieties are susceptible to three of these forms; the durums are resistant to forms 36 and 49, susceptible to form 21, and under favorable environmental conditions they are susceptible to form 38, which causes a type x+ infection. Reliance is immune from forms 21 and 49 but is susceptible to forms 36 and 38 (table 5). This variety reacts like Kanred, which often is immune in the field. It rusts heavily, however, when forms like 36 and 38 are present. Kota, which reacts like the widely grown variety, Ceres, is at least moderately susceptible to all four forms but seldom becomes heavily rusted in the field because of morphologic resistance.

Form 48 has never been found in the United States except near rusted barberries, although it has been reported several times from Canada, where it was first collected in 1929, on wheat. It is, however, of rare occurrence.

PUCCINIA GRAMINIS SECALIS

The physiologic forms of 27 aecial collections of *Puccinia graminis secalis* were identified, 9 forms being found. It seems significant that so many forms were isolated from so few collections. On an average, a different form was isolated from every third collection. Forms 7 and 11 were the most prevalent, constituting 25.93 and 29.63 percent, respectively, of the total (tables 6 and 8, and figs. 1 and 2).

TABLE 5.—*Types of infection*[a] *on differential varieties of wheat caused by physiologic forms of Puccinia graminis tritici isolated from aecia and from uredia near infected barberries, 1919–32*

Form	Reaction of differential varieties											
	Jenkin	Marquis	Reliance	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
1	4	1	0	3	1	1	1	3	3	3	0	1
2	4	2	2	2	1	1	1	1	3	3	1	0
3	4	4	4	3	1	1	1	1	3	3	1	0
9	4	4	0	3	4	4	4	1	3	3	4	1
11	4	4	3	3	4	4	4	3	3	3	1	1
12	4	4	4	3	1	1	1	1	3	3	1	0
15	4	1	4	3	4	4	4	3	3	3	4	1
17	4	4	0	3	4	4	4	3	3	3	1	1
18	4	4	4	3	1	1	1	3	3	3	1	1
19	4	2	0	3	4	4	4	3	3	3	0	1
21	4	4	0	3	4	4	4	1	3	1	0	1
23	4	2	1	1	1	1	1	3	3	3	0	0
24	4	1	0	2	1	1	4	3	3	3	1	0
29	4	4	0	3	x	x	x	x	x	3	1	1
32	4	1	1	3	x	x	x	x	x	3	1	1
33	4	2	1	1	1	1	1	1	3	3	1	1
34	4	1	1	4	1	1	1	1	3	1	0	1
36	4	4	1	3	1	1	0	x	3	3	0	1
37	4	4	0	3	4	4	4	x	3	3	1	1
38	4	2	4	3	x	x	x	x	x	4	1	1
39	4	2	4	3	4	3	4	4	3	4	1	1
48	4	1	0	1	x	x	x	x	1	1	1	1
49	4	1	0	1	1	1	0	x	3	1	0	1
51	4	2	3	0	0	0	0	4	3	3	4	0
52	4	4	1	1	1	1	1	x	4	4	1	1
56	1	3	3	3	1	1	1	3	3	1	1	1
61[b]	4	4	0	3	0	0	0	x	4	4	0	0
62[c]	4	x	x	x	4	4	4	4	4	3	0	1
66[b]	4	2	4	0	0	0	0	x	1	3	0	0
67[d]	4	4	4	4	1	2	2	x	x	3	4	1
96[d]	4	x	4	x	4	4	1	1	3	3	1	1
101	4	4	1	4	1	0	0	x	x	3	0	1
102[c]	4	0	1	0	0	0	1	1	0	3	0	1
104[c]	4	x	0	0	0	0	0	0	0	3	x	0
105[c]	4	x	0	3	0	0	0	x	3	3	x	1
125	4	4	4	4	0	0	0	x	4	1	0	1
127[d]	4	4	3	3	1	1	1	x	x	0	0	1

[a] 0 signifies immune, 1, very resistant; 2, moderately resistant, 3, moderately susceptible, 4, very susceptible; x, heterogeneous (uredia very variable, various types of infection appearing on the same leaf, not due to mechanical mixture.

[b] First isolated from barberries.

[c] Isolated only from barberries

[d] From selfing only.

TABLE 6.—*Physiologic forms of Puccinia graminis secalis and P. graminis avenae, isolated from aecial collections, by States, 1920–32*

NUMBER OF TIMES FORM WAS FOUND

Variety and form	Kansas	Maine	Michigan	Minnesota	Missouri	Nebraska	South Dakota	Wisconsin	Total
P. graminis secalis									
3				1					1
4				1					1
5		1							1
7				6				1	7
8				1					1
9				3					3
11	3		2	2			1		8
12				3		1			4
14							1		1
Total	3	1	2	17		1	2	1	27
P. graminis avenae									
2	1		1	2					4
5	1				1				2
Total	2		1	2	1				6

NUMBER OF FORMS

Variety and form	Kansas	Maine	Michigan	Minnesota	Missouri	Nebraska	South Dakota	Wisconsin	Total
P. graminis secalis	1	1	1	7		1	2	1	9
P. graminis avenae	2		1	1	1				2

Form 5 is the only form that had not been isolated previously from uredial material. As it was subsequently obtained also from uredia near barberries, it probably had been formed recently.

From 28 uredial collections of *Puccinia graminis secalis* near infected barberries, 8 physiologic forms were isolated, again a large number of forms in proportion to the number of collections. Forms 7 and 11 were again the most prevalent (table 7).

PUCCINIA GRAMINIS AVENAE

Only six aecial collections of *Puccinia graminis avenae* were identified. Forms 2 and 5 occurred in the ratio of 2 to 1. Both are extremely common and widely distributed in the United States; consequently no particular significance attaches to their occurrence on barberries. However, an entirely new form, designated form 10, was obtained from oats near rusted barberries in Wisconsin. A special note on this form has been published by Cotter (*2*); hence details will not be repeated here, beyond calling attention to the fact that it may reasonably be concluded to have resulted from hybridization on barberries in nature. It is important to note that form 10 is much more virulent on the Richland group of oat varieties than either form 2 or form 5 (table 8).

TABLE 7.—*Physiologic forms of Puccinia graminis secalis and P. graminis avenae isolated from uredial collections made near infected barberries, by States, 1919–32*

NUMBER OF TIMES FORM WAS FOUND

Variety and form	Illinois	Iowa	Kansas	Maine	Michigan	Minnesota	Missouri	Nebraska	New York	Ohio	South Dakota	Wisconsin	Total
P. graminis secalis:													
2												1	1
3	2					1						1	4
5					1								1
7			3		1	3	1						8
8			2	1		1							4
9								1					1
11	1	1	1		2	1			1		1		8
12												1	1
Total	3	1	6	1	4	6	1	1	1		1	3	28
P. graminis avenae:													
2		1	1			1				1		1	5
5						2				1			3
10												2	2
Total		1	1			3				2		3	10

NUMBER OF FORMS

	Illinois	Iowa	Kansas	Maine	Michigan	Minnesota	Missouri	Nebraska	New York	Ohio	South Dakota	Wisconsin	Total
P. graminis secalis	2	1	3	1	3	4	1	1	1		1	3	8
P. graminis avenae		1	1			2				2		2	3

TABLE 8.—*Types of infection produced on differential varieties of rye and oats caused by physiologic forms of Puccinia graminis secalis and P. graminis avenae, respectively, isolated from aecia and from uredia near infected barberries, 1920–32*

Form	Varieties of rye and relative susceptibility to *P. graminis secalis*					Varieties of oats and mean infection type		
	Rosen	Swedish	Prolific	Dakold	Colorless	Minrus	Richland	Joanette
2	88.1	15.8	40.0			2	1	4
3	88.5	83.4	81.4	60.9	88.3			
4	93.3	81.8	62.6	61.3	96.9			
5	84.3	6.9	85.7			2	1	x
7	89.3	62.7	60.4	38.0	88.8			
8	67.8	41.4	62.1	13.6	79.4			
9	87.3	52.4	81.2	19.6	86.7			
10						2	4	x
11	87.4	46.7	58.7	17.4	86.6			
12	86.1	63.5	81.2	36.7	87.8			
14	85.8	39.9	53.9	10.8	68.3			

PHYSIOLOGIC FORMS FROM AECIA RESULTING FROM SELFED PYCNIA

To obtain still further information on the role of barberries in producing new physiologic forms and in perpetuating old ones, a number of "selfings" were made. The method was to inoculate barberries with collections of telial material, then transfer pycnial nectar from certain pycnia to others of the same collections, and inoculate differential hosts with the progeny of spores resulting from inoculations with spores from single aecia. There is, of course, no way of knowing certainly whether forms isolated were already present in the telial stage, whether they resulted from segregation, or whether new combinations were made. The essential fact brought out by the results, however, is that in proportion to the number of cultures isolated a large number of forms was always obtained. From some of the aecia resulting from inoculation with teliospores, 2 forms, and in one case, 4 forms, were obtained. The average ratio between the number of forms isolated and the number of telial collections used for inoculation was 3 to 2. The ratio was approximately the same with *Puccinia graminis tritici* and *P. graminis secalis*, being 1.62 to 1 and 1.56 to 1, respectively. As has been previously pointed out, when determinations of physiologic forms of *P. graminis tritici* were made from uredial material collected at random, one physiologic form was isolated from approximately every 100 collections. From aecial material resulting from inoculating barberries with teliospores in the greenhouse, however, the average number of forms isolated from each collection was 0.39. This would seem to indicate, again, that the eradication of barberries will result in a diminution of the number of forms.

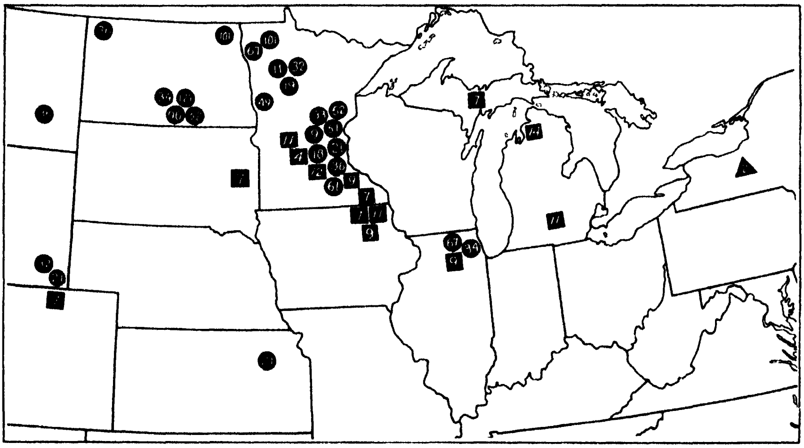

FIGURE 3.—Geographic distribution of physiologic forms (designated by number) of *Puccinia graminis*, segregated after passing through susceptible barberry. Circles indicate variety *tritici*; squares, *secalis*; triangle, *avenae*.

From a total of 30 cultures of *Puccinia graminis tritici*, 17 different physiologic forms were isolated (table 10). Those obtained most commonly were forms 67 and 101, not forms 36 and 38, which were most frequently isolated from aecial collections and from uredial collections made near barberries in nature. Nevertheless, form 36 follows forms 67 and 101 in order of frequency and is very similar to them in pathogenicity, but form 38 was not obtained at all. The complete list of forms isolated is given in table 10. (See also fig. 3.)

Forms 67, 96, and 127, which were isolated in these experiments, have never been obtained from uredial material. Of these, forms 67 and 96 had previously been produced as a result of artificial hybridization and form 127 was first isolated as a result of selfing but has been produced subsequently by hybridization also. Form 101 had been identified from uredial material collected in Bulgaria but has not been found in nature in North America. Its only recorded appearance on this continent is on barberries on which pycnia were selfed.

Forms 7, 9, 11, and 12 of *Puccinia graminis secalis* also were isolated from aecia produced on barberries in nature and from uredia obtained near barberry bushes. Of these, 7 and 11 have been most prevalent throughout. Forms 4 and 14 were obtained once each from the aecial material and from the products of selfing, but form 14 has never been obtained from uredial isolations.

It seems probable from the results given above that still other new forms might be produced if there were opportunity for certain telial material to cause infection on barberries in nature.

Table 9.—*Physiologic forms of Puccinia graminis varieties isolated from aecia produced as a result of selfing pycnia on barberries inoculated with collections of teliospores, 1926–32*

Year	Number of selfings[a] and number of forms isolated of *P. graminis*							
	Total		Var. *tritici*		Var. *secalis*		Var. *avenae*	
	Selfings	Forms isolated	Selfings	Forms isolated	Selfings	Forms isolated	Selfings	Forms isolated
1926	1	4	1	4				
1927	2	5	1	2	1	3		
1928	1	1	1	1				
1929	5	7	2	2	3	5		
1930	2	3	1	1	1	2		
1931	6	7	4	5	2	2		
1932	14	21	11	19	2	2	1	0
Total	31	48	21	34	9	14	1	0
Ratio of number of forms to selfings	1.55:1		1.62:1		1.56:1		0:1	

[a] This indicates the number of times barberries were inoculated with teliospore collections. Several pycnia were selfed in each case and inoculations made with aeciospores from the resulting aecia. Urediospores were then used for inoculating differential varieties.

DISCUSSION AND CONCLUSIONS

The results presented in the foregoing pages show clearly that barberries are important in two ways besides that of producing abundant inoculum early in the spring: (1) They enable new physiologic forms to arise through hybridization, and (2) they apparently enable many forms to persist and multiply.

The results of these field studies support the opinion, based on previous greenhouse experiments, that new forms arise on barberries in nature. Four forms of *Puccinia graminis tritici* have been isolated only from naturally infected barberries in the field, and two others were isolated from barberries before they were found elsewhere. Form 5 of *P. graminis secalis* has been isolated only from aecia on barberries and from uredia in close proximity to infected barberries, while form 10 of *P. graminis avenae* has been found only near infected barberries. The writers are convinced that these forms were of recent origin, indicating that new forms are still arising on susceptible barberries. The significance of the results is increased by the fact that these forms had not been found previously in extensive physiologic-form surveys made each year since 1917.

There is strong circumstantial evidence that the numerous physiologic forms now in existence have arisen principally as a result of hybridization. Despite very extensive experiments with physiologic

TABLE 10.—*Physiologic forms of Puccinia graminis varieties isolated from aecia produced as a result of selfing pycnia on barberries inoculated with collections of teliospores, by States, 1926-32*

NUMBER OF TIMES FORM WAS FOUND

Variety and form	Colorado	Illinois	Iowa	Kansas	Michigan	Minnesota	Montana	New York	North Dakota	South Dakota	Wyoming	Total
P. graminis tritici												
9						1						1
11						1						1
18						1						1
19						1						1
21				1		1					1	3
32						1					1	2
33						1						1
34		1							1			2
36						1	1		1			3
49						1						1
51						1						1
52						1			1			2
61						1						1
67		1				3						4
96									1			1
101						3			1			4
127									1			1
Total		2		1		18	1		6		2	30
P. graminis secalis												
4						1						1
7	1		1		2	4				1		9
9		1	1			1						3
11			1		1	12						14
12						1						1
14					1							1
Total	1	1	3		4	19				1		29
P. graminis avenae												
2								1				1

NUMBER OF FORMS

Variety and form	Colorado	Illinois	Iowa	Kansas	Michigan	Minnesota	Montana	New York	North Dakota	South Dakota	Wyoming	Total
P. graminis tritici		2		1		14	1		6		2	17
P. graminis secalis	1	1	3		3	5				1		6
P. graminis avenae								1				1

forms since 1916, the writers have observed only two definite cases of mutation in parasitism, which are believed to be the only ones on record for *Puccinia graminis*. Color mutations are not infrequent, but mutation in pathogenicity seems rare. On the other hand, there is abundant evidence that new forms arise frequently through hybridization. It seems reasonable, therefore, to assume that most physiologic forms have arisen in this way.

The large number of forms in regions where barberries become heavily rusted and the smaller number in regions where the aecial stage is rare also support the hypotheses just stated. As already pointed out by Waterhouse(*13*), the presence of a large number of forms in the Mississippi Valley of North America and of a small number in Australia seems significant. Furthermore, in the rather isolated "Inland Empire" of the Pacific Northwest of the United States, where barberries rarely become rusted, there seem to be relatively few forms of *Puccinia graminis tritici*, although wheat has long been grown extensively in the region and club wheats, which are susceptible to nearly all forms known in North America, have been grown commonly.

It is a striking fact, also, that so large a number of forms can be isolated from aecia and from uredia on grains and grasses in close proximity to barberry bushes. For example, a different form of *Puccinia graminis tritici* was isolated from approximately every third collection of aecial material, whereas a different form was isolated from only each 100 collections of uredial material collected at random (tables 11 and 12).

TABLE 11.—*Summary of the identity and number of physiologic forms of Puccinia graminis varieties isolated from aecial and telial collections, and uredial collections made near barberries, 1920–32*

Form	Number of times each form was found							
	Aecial collections from infected barberries			Uredial collections near infected barberries			Telial collections selfed on barberries	
	Var. *tritici*	Var. *secalis*	Var. *avenae*	Var. *tritici*	Var. *secalis*	Var. *avenae*	Var. *tritici*	Var. *secalis*
1	6							
2	2		4	1	1	5		
3	1	1			4			
4		1						1
5		1	2		1	3		
7		7			8			9
8		1			4			
9	2	3		2	1		1	3
10[a]						2		
11	4	8		3	8		1	14
12		4		1	1			1
14		1						1
15	2							
17	4			1				
18	1			4			1	
19	3			2			1	
21	5			9			3	
23				1				
24	1							
29	2			1				
32	3			1			2	
33				2			1	
34	2						2	
36	24			16			3	
37				1				
38	11			14				
39	5			1				
48				1				
49	6			9			1	
51							1	
52	1						2	
56				1				
61[b]	2						1	
62[c]	1							
66[b]	1							
67[d]							4	
96[d]							1	
101							4	
102[c]	1							
104[c]	1							
105[c]	2							
125	1							
127[d]							1	
Total	94	27	6	71	28	10	30	29
Number of forms	26	9	2	19	8	3	17	6

[a] Found near infected barberries only.
[b] First isolated from barberries.
[c] Isolated only from barberries.
[d] From selfing only.

TABLE 12.—*Physiologic forms isolated as compared with collections of Puccinia graminis varieties cultured*

Variety	Physiologic forms isolated from collections of designated spore stages							
	Aecial		Uredial		Telial		Weighted average [a]	
	Ratio [b]	Percent [c]	Ratio [b]	Percent [c]	Ratio [b]	Percent [c]	Ratio [b]	Percent [c]
P graminis tritici	3.62:1	27.66	3.74:1	26.76	1.76:1	56.67	3.15:1	31.72
P graminis secalis	3.00:1	33.33	3.50:1	28.57	4.83:1	20.68	3.65:1	27.38
P graminis avenae	3.00:1	33.33	3.33:1	30.00	--		3.20:1	31.25
Weighted average for spore stages	3.43:1	29.13	3.63:1	27.52	2.57:1	38.98	3.28:1	30.51

[a] As a basis of comparison, the ratio for *P graminis tritici* was approximately 100:1 in the general physiologic-form survey, when collections were made at random in the United States and Mexico, that is, from approximately each 100 collections a different form was obtained

[b] Ratio of number of collections cultured to number of physiologic forms found

[c] Percentage of physiologic forms in terms of the number of collections cultured

Evidently barberries are responsible, therefore, not only for the production of new forms, but also to a considerable extent for their persistence; and in previous publications abundant evidence has been presented showing that barberries also are responsible for the dissemination of a tremendous amount of inoculum early in the growing season.

SUMMARY

The writers have investigated the role of barberries in the production and perpetuation of physiologic forms of *Puccinia graminis* in nature.

During the past 13 years inoculations were made on the common small grains with material from 675 aecial collections of *Puccinia graminis* obtained from the northern part of the United States. Of these, 281 caused infection, 34.2 percent being of the *tritici* variety, 63.7 percent of the *secalis* variety, and only 2.1 percent of the *avenae* variety.

The relative prevalence of the different varieties of *P. graminis* probably is governed to a considerable extent by the distribution of wild grasses susceptible to the different varieties.

The varietal identity of 138 uredial collections of *P. graminis* obtained within 100 yards of rusted barberries also was determined, with the following percentages: *tritici*, 52.2 percent; *secalis*, 32.6 percent; and *avenae*, 15.2 percent. These percentages probably were affected by a certain amount of conscious selection of hosts known to be susceptible to certain rust varieties.

The results given above, supplemented by other observations, indicate that stem rust of rye (*P. graminis secalis*) is almost wholly dependent on barberries for its persistence in the United States.

From 94 aecial collections of *P. graminis tritici*, 26 physiologic forms were isolated, a different form from approximately every 4 collections, whereas from about 8,000 uredial collections made at random over a period of years a different form was isolated from about every 100 collections.

Of the physiologic forms isolated from *P. graminis tritici*, forms 36 and 38 were the most prevalent.

Four forms (62, 102, 104, and 105) never have been isolated from any source other than rusted barberries. Forms 61 and 66 were isolated first from rusted barberries but subsequently were isolated from rusted wheat also.

From 71 uredial collections of *P. graminis tritici* made near rusted barberries, 19 physiologic forms were obtained, one of which (form 48) never has been found elsewhere in the United States, although reported several times from Canada.

The results indicate that barberries in nature are responsible for the production of new physiologic forms of *P. graminis tritici* as well as for the persistence of numerous forms.

Twenty-seven aecial collections of *P. graminis secalis* comprised 9 physiologic forms, and 28 uredial collections obtained near rusted bushes comprised 8 forms, a far larger number in proportion to the number of collections than in the case of uredial collections made at random. Forms 7 and 11 were the most prevalent; form 5 has been obtained only from aecia or uredia formed near infected barberries, and form 14 was isolated only from aecial collections or was the product of "selfing" on barberry.

Only six aecial collections of *P. graminis avenae* were identified, forms 2 and 5, which are widely distributed in the United States, being isolated. A new form (10), however, was isolated from oats near rusted barberries. It is far more virulent on the Richland group of oat varieties than either of the other two forms mentioned.

When barberries were inoculated with telial material in the greenhouse, the ratio between the number of forms isolated and the number of telial collections used for inoculating was 2 to 5. For example, 17 forms were isolated from 30 cultures of *P. graminis tritici* and 6 forms from 29 cultures of *P. graminis secalis*.

Forms 67, 96, and 127 of *P. graminis tritici*, isolated as a result of inoculating barberries with teliospores, never have been obtained from uredial material in the field, and another form (101) has never been found in the United States except on artificially inoculated barberries, although it was isolated from uredial material collected in Bulgaria.

Two physiologic forms (4 and 14) of *P. graminis secalis* were isolated from artificially inoculated barberries and from naturally infected bushes in the field, but form 14 has never been isolated from uredial material.

It is concluded that in nature barberries are important in the production and persistence of physiologic forms and in the persistence of certain varieties of rust, especially *P. graminis secalis* and probably *P. graminis agrostidis* and *P. graminis poae* also.

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SIZE AND ARRANGEMENT OF PLOTS FOR YIELD TESTS WITH CULTIVATED MUSHROOMS[1]

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INTRODUCTION

Numerous studies have been made to determine the most efficient plot technic for various agronomic and horticultural crops under different conditions. A bibliography on this subject has been published recently.[3]

In the few papers dealing with the results of yield tests in experiments with the cultivated mushroom (*Agaricus campestris* L.) a discussion of plot technic has been entirely neglected. Yet plot technic is of unusual importance in mushroom tests since there is normally more variability in mushroom beds than in ordinary field plots and the experimenter has an unusual opportunity to control conditions. Furthermore, the number and size of experimental plots must be reduced to a minimum because the labor and expense of yield tests is increased tremendously by the mushroom's habit of fruiting continuously for 3 or 4 months.

In commercial practice mushrooms are usually grown on shelf beds in windowless sheds or houses. An average mushroom house is about 60 feet long and contains 2 tiers of 5 or 6 shelf beds in which the individual beds are 24 inches apart. The beds are 5 or 6 feet wide and run the entire length of the house, except for a narrow passageway at each end of the house.[4] As a result of the placing of uprights and bed supports at 4-foot intervals along the bed (fig. 1), the beds are normally subdivided into small sections 4 feet wide and extending across the bed.

These structural features suggest at least four convenient units of bed space which might be used for yield trials—entire houses, tiers of beds, beds, and sections.

Practical experience soon shows that entire houses are not suitable, for differences in compost heaps and in the prevalence of fungus diseases and pests in the various houses more than counterbalance the advantages gained by the large area harvested. Tiers of beds are preferable to entire houses, since comparisons may be made among test areas located in the same house, and since the beds are made from the same compost heap. For most experiments, however, this arrangement is not satisfactory because it precludes replication of the experimental areas owing to the fact that there are only two tiers of beds in a house. These objections do not apply to the use of beds

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[2] The writer gratefully acknowledges his indebtedness to L F. Lambert and L R Downing, who conducted the yield tests at Coatesville, Pa, and Downingtown, Pa, respectively, to L R Fate, Division of Mycology and Disease Survey, for assistance in the arithmetical calculations, and to F R Immer, Division of Sugar Plant Investigations, for helpful suggestions pertaining to the analysis of the data

[3] GARBER, R. J., LOVE, H H, MOOERS, C. A., and KIESSELBACH, T A. STANDARDIZATION OF FIELD EXPERIMENTS. Jour. Amer. Soc. Agron 22 1056–1061. 1930

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or smaller plots; such as sections or groups of sections, for test units. Therefore, for most experiments beds or parts of beds would seem to be, a priori, the proper experimental units.

The choice between entire beds and smaller plots depends largely on the comparative variability of the yields from these units. The experiments outlined in this paper were designed primarily to throw light on this question and on the problem of plot arrangement.

MATERIAL AND METHODS

The experiments were conducted as simple uniformity trials. They were made in the Department's experimental mushroom cellar at the Arlington Experiment Farm, Rosslyn, Va., and in commercial houses at Downingtown and Coatesville, Pa. The manure was composted in the regular manner, and the beds were filled with compost at the rate of approximately 1 bushel for 2 square feet of bed space. At Arlington Farm and Downingtown the beds were filled by emptying 10 bushel baskets into each 4-foot section on beds 5 feet wide and 12 bushel baskets into each section on beds 6 feet wide. At Coatesville 2 sections were filled at one time with 24 bushels of compost. In each experiment the composting in the heap was done as uniformly as possible, the same batch of spawn was used for the entire surface, and the casing soil was from a single source in each house. The sections at the ends of the beds were discarded because the yields from these sections are frequently reduced by excessive drafts. The picking was done at intervals of from 1 to 3 days until the beds were practically exhausted. In this connection it should be noted that the production period was terminated at Arlington Farm by a spell of hot weather

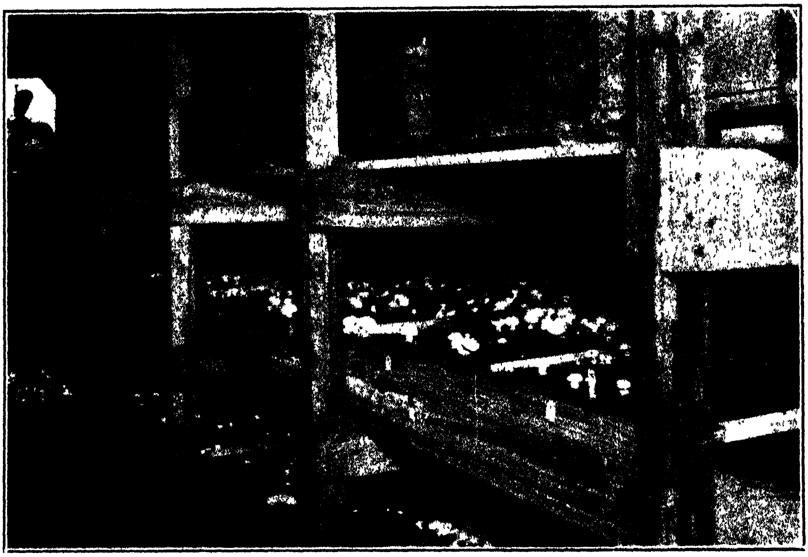

FIGURE 1.—Experimental mushroom beds at Arlington Experiment Farm, Rosslyn, Va., divided into ½-section plots 2 feet wide and extending across the bed. Note the placing of upright supports at 4-foot intervals, this is the standard arrangement in commercial houses and the beds as a matter of convenience are usually filled, with sections as unit areas, by unloading 10 or 12 bushels of compost at one time in the area between upright supports.

and the lower beds, which were slow to start, were injured more than the upper beds.

At Arlington Farm the total experimental area consisted of 5 beds each divided into 10 plots one half section (2 feet) wide (fig. 1). At Downingtown 5 beds were each divided into 10 plots one section (4 feet) wide. At Coatesville 4 beds were each divided into 10 plots one section (4 feet) wide. The yields given in table 1 for the separate plots are the sums of all the daily yields taken over a period of 3 months for each plot.

The "analysis of variance" method, devised and described by Fisher,[5] was used for analyzing the data. An arithmetical procedure was followed similar to that outlined by Fisher and Wishart.[6]

ANALYSIS OF YIELD DATA FROM UNIFORMITY TRIALS

ARLINGTON EXPERIMENT FARM, ROSSLYN, VA.

The yields of the 50 individual plots on the 5 experimental beds at Arlington Farm are given in table 1. If the 10 plots are considered as separate treatments and the beds as replicate blocks of these treatments, an analysis of variance, as shown in table 2, may be calculated that helps to clear up many of the points with which we are concerned.

TABLE 1.—*Yields of mushrooms in 3 uniformity trials*

YIELD PER 10-SQUARE-FOOT PLOT IN EXPERIMENTAL HOUSE AT ARLINGTON EXPERIMENT FARM, ROSSLYN, VA

Bed no	Yield from plot no 1	2	3	4	5	6	7	8	9	10
	Ounces	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*	*Ounces*
1	382	263	242	312	184	226	283	273	272	262
2	286	338	239	336	239	297	216	231	220	266
3	153	154	143	175	167	203	209	170	203	201
4	145	143	158	170	176	174	127	167	133	153
5	102	148	158	194	176	181	183	239	210	193

YIELD PER 24-SQUARE-FOOT PLOT IN A COMMERCIAL MUSHROOM HOUSE AT DOWNINGTOWN, PA

Bed no	1	2	3	4	5	6	7	8	9	10
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	21	21	25	24	28	30	20	24	25	24
2	36	27	32	27	28	27	24	38	30	23
3	20	20	20	29	15	24	36	29	21	30
4	28	25	16	22	23	24	20	21	20	27
5	24	21	19	36	26	33	28	24	32	23

YIELD PER 24-SQUARE-FOOT PLOT IN A COMMERCIAL MUSHROOM HOUSE AT COATESVILLE, PA

Bed no	1	2	3	4	5	6	7	8	9	10
1	31	36	42	37	50	45	42	40	43	37
2	42	41	41	42	41	31	45	47	45	42
3	43	40	41	40	40	50	37	45	46	39
4	31	34	42	35	42	37	41	41	41	43

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[5] FISHER, R A STATISTICAL METHODS FOR RESEARCH WORKERS Ed 3, rev and enl, 283 p, illus Edinburgh and London 1930

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TABLE 2.—*Analysis of variance of yields of mushrooms harvested in 3 locations from plots of different sizes and arrangement*

ARLINGTON EXPERIMENT FARM, ROSSLYN, VA. (YIELD IN OUNCES)

Arrangement of plots corresponding to fig. 2	Plots: Width	Plots: Per block	Plots: Area of each (sections or parts of sections)	Blocks or beds (total area)	Variation	Degrees of freedom [a]	Sum of squares	Variance	z [b]	Standard deviation [c]	Coefficient of variability of— Single plot	Coefficient of variability of— Mean of 5 replications [d]	Lowest significant difference (per cent between means of 5 replications) [e]	Efficiency (per cent on an area basis) [f]
	Feet	*Number*		*Number*										
A	2	10	1	5	Between beds	4	115 731.6	28 932.90	1.5440	36.32	17.24	7.71	23.13	100
					Between columns	9	10 432.9	1 159.21						
					Interaction	36	47 486.0	1 319.06						
					Total	49	173 650.5							
B	4	5	1	5	Between beds	4	231 463.5	57 865.87	1.4316	57.47	13.6	6.08	18.24	80.3
					Within beds	20	66 058.5	3 302.92						
					Total	24	297 522.0							
C	6	3	1½	5	Between beds	4	322 002.0	85 504.4	1.3168	78.35	12.4	5.54	16.62	68.3
					Within beds	11	67 543.0	6 140						
					Total	15	389 545.0							
D	4	4	2 half	5	Between bed	4	165 479.3	41 369.82	1.6733	39.41	9.57	4.28	12.84	162.2
					Within beds	15	23 297.5	1 553.16						
					Total	19	188 776.8							

COMMERCIAL MUSHROOM HOUSE AT DOWNINGTOWN, PA. (YIELD IN POUNDS)

E	4	10	1	5	Between beds	4	261.6	65.40	0.47468	5.01	19.7	8.81	26.43	100
					Between columns	9	154.4	17.15						
					Interaction	36	906.0	25.17						
					Total	49	1,322.0							
F	8	5	2	5	Between beds	4	523.0	135.8	.47328	7.26	14.8	6.62	19.86	88
					Between columns	4	50.8	12.7						
					Interaction	16	864.0	52.7						
					Total	24	1,437.8							

COMMERCIAL MUSHROOM HOUSE AT COATESVILLE, PA. (YIELD IN POUNDS)

G	4	10	1	4	Between beds	3	74.2	24.9	-----	4.3	10.6	4.74	14.22	100
					Between columns	9	214.4	23.8						
					Interaction	27	500.8	18.5						
					Total	39	789.4							
H	8	5	2	4	Between beds	3	134	44.5	-----	5.9	7.2	3.22	9.66	108
					Between columns	4	281	70.2						
					Interaction	12	488	37.3						
					Total	19	903							
I	8	4	2	4	Between beds	3	100.7	33.90	-----	4.23	4.1	1.8	5.5	334
					Between columns	3	126.5	42.16						
					Interaction	9	160.8	17.87						
					Total	15	388.0							

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[a] Number of independent comparisons.

[b] Sum of the squares of the deviations from the mean yield.

[c] Variance or mean square $=\frac{\text{sum of squares}}{\text{degrees of freedom}}$.

[d] z = one half of the difference between the natural logarithms of the mean squares between beds and of error.

[e] Standard error (standard deviation) = square root of variance.

[f] Coefficient of variability = standard error in percentage of the mean yield $=\frac{\text{S. E.}\times 100}{\text{mean yield}}$.

[g] Coefficient of variability divided by the square root of 5.

[h] $\frac{3\times \text{CV}}{\sqrt{5}}$. (Odds approximately 20 to 1 against the occurrence due to chance of a difference as great or greater.)

[i] See p. 976.

In the reduction of the data the first objective was to determine the comparative efficiency of beds taken as a whole and of smaller plots such as sections or groups of sections. The data indicate higher variability between beds than between smaller plots (within beds) and lend themselves to the application of Fisher's z test to determine the statistical significance of this difference. When Fisher's table VI is consulted it is apparent that the observed value of z exceeds the 1-percent point in all cases, and it may be concluded that the differences are significant. The odds are more than 100 to 1 that such a difference would not occur as a result of chance variation. Since the variance between beds was significantly greater than the variance within beds, the data indicate that greater precision can be expected with smaller plots, and justify the arrangement of plots in the beds so that the variance between beds can be eliminated in calculating the standard error. In practice this would mean the random distribution of treatments on the beds with the restriction that each treatment shall occur once on each bed.

Conceivably, there might be conditions in the mushroom house that would bring about a consistently larger yield at one end of several of the beds analogous to the fertility gradients frequently encountered in soil-heterogeneity studies. Under these conditions there probably would be justification for arranging the plots in 5 by 5 Latin squares, in which the plots in each bed would constitute the rows and the plots one above the other or opposite one another in the house would constitute the columns of the square. Since all the plots have been treated uniformly the justification for such a procedure can be tested from the data in table 1 by calculating and comparing the variance between columns with the variance within columns and blocks. This comparison also is given in the analysis of variance shown in table 2. In this case the variance due to the interaction of beds and columns (error) is larger than the variance between columns, indicating that there would be no significant gain in the precision of the experiment by arranging the plots in a Latin square.

The next point of interest to be considered is the gain or loss in efficiency from the use of different-sized plots, such as one-half section (10 square feet), full section (20 square feet), or 1½ sections (30 square feet). In a series of analysis of variance as shown in table 2 the standard error in percentage of the mean, or coefficient of variability, was found to be reduced from 17.24 for one-half sections to 13.6 for entire sections, and 12.4 for 1½ sections (triple plots). On a plot basis this indicates a gain in precision with increase in plot size, but full-section plots take up twice as much space as the ½-section plots and 1½-section plots take up three times as much space. This raises the question of the relative efficiency of ½-section plots, full-section plots, and triple plots when the same area is used in each case. Immer[7] gives a method of determining the efficiency of plots of varying size and shape, calculated on the basis of variance per unit area of land, that can be used to answer this question. With the small plot as a standard, the relative efficiency of the larger plots was found by multiplying the square of the coefficient of variability per plot by the quotient of the area of the larger plot divided by the area of the small plot and expressing the result in percentage of the square of the coefficient of variability of the small plot used as a standard. When calculated in this way, if the ½-section plot is considered as 100 percent efficient it becomes apparent that on an area basis the efficiency of the full section is only 80.3 percent and the efficiency of the 1½-section plot only 68.3 percent. In other words, under the conditions exemplified by the plots studied, 10 replications of ½-section plots are preferable to 5 replications of entire sections; and 15 replications of ½-section plots are preferable to 5 replications of 1½-section plots.

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[7] IMMER, F. R. SIZE AND SHAPE OF PLOT IN RELATION TO FIELD EXPERIMENTS WITH SUGAR BEETS. Jour. Agr. Research 44: 649–668, illus. 1932.

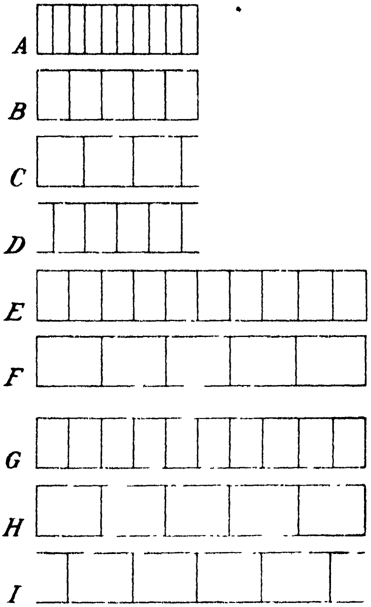

FIGURE 2.—Diagram showing relative size and arrangement of plots in typical blocks. *A–D*, Plots at Arlington Experiment Farm, Rosslyn, Va.: *A*, 2-foot plots, 10 plots per block (or bed), 5 blocks (total area); *B*, 4-foot plots, 5 plots per block, 5 blocks; *C*, 6-foot plots, 3 plots per block, 5 blocks; *D*, 4-foot plots, 4 plots per block, overlapping units of filling, 5 blocks. *E* and *F*, plots at Downingtown, Pa.: *E*, 4-foot plots, 10 plots per block, 5 blocks; *F*, 8-foot plots, 5 plots per block, 5 blocks. *G–I*, plots at Coatesville, Pa.: *G*, 4-foot plots, 10 plots per block, 4 blocks; *H*, 8-foot plots, 5 plots per block, 4 blocks; *I*, 8-foot plots, 4 plots per block, overlapping units of filling, 4 blocks.

Theoretically it seems probable that the customary method of filling the beds with compost and of using sections in the beds as units for filling would cause a greater variability between normal sections than between areas of similar size that do not coincide with the sections on the bed. The data at hand offer a means of testing this hypothesis. It is merely necessary to shift over one plot before pairing the yields of the ½-section plots to obtain for consideration a set of yields from areas equal in size to sections but overlapping the normal sections in the bed. If the foregoing conjecture is correct the coefficient of variability calculated from these units of area should be lower than the coefficient of variability calculated when normal sections are used as plots. An analysis of variance based on 4 shifted plots in each of 5 beds, as shown graphically in figure 2, gave a coefficient of variability of 9.57 in contrast to the coefficient of variability of 13.6 obtained for normal sections. The conclusion, therefore, seems justified that less variability may be expected when the experiment is so arranged that the areas used as units for harvesting do not coincide with the areas used as units for filling the beds with compost. On an area basis the overlapping plots were 201.9 percent as efficient as the normal sections and 162.2 percent as efficient as the ½-section plots.

DOWNINGTOWN, PA.

The yields of 50 plots from the five experimental beds at Downingtown, Pa., are given in table 1.

When the analysis of variance summarized in table 2 is applied to these data the value of z obtained from a comparison of the variance within beds with the variance between beds is 0.47468. This is slightly less than the 5-percent point, and indicates that, unlike the Arlington Farm experiments, the precision of the experiment was not significantly increased by the arrangement of the plots to facilitate elimination of the variability between beds. In like manner it was shown that there is no justification for arranging the experiment in Latin squares and removing variability due to columns. On an area basis, however, there can be little doubt of the advantage of using replicate small plots rather than entire beds.

The coefficient of variability, calculated from the total variance for the data obtained by harvesting plots coinciding with sections on the bed (table 2), is 19.7. When the area of the plot is doubled (fig. 2 and table 2) the coefficient of variability drops to 14.8. The lower coefficient of variability is to be expected both on the basis of the increase in the size of plot and the dissimilarity of the area used for harvesting and the area used for filling the bed with compost. However, on an area basis the double section is only 88 percent as efficient as the section plot.

COATESVILLE, PA.

The yields of 40 plots from the 4 experimental beds at Coatesville, Pa., are given in table 1.

An analysis of variance applied to these data leads to conclusions similar to those derived from the experiments at Downingtown. Here again it is evident that small plots are preferable to entire beds on an area basis although there was no significant gain in the precision of the experiment by accounting in the analysis for the variance between beds or between columns. The coefficients of variability calculated from the total variance for single sections and double sections, respectively, were 10.6 and 7.2 (table 2). This experiment differed from the previous ones in that on an area basis the double section was slightly more efficient (108 percent) than the single section.

It should be recalled that in this experiment the units used for filling the beds with compost were double sections corresponding to plots 1 and 2, 3 and 4, 5 and 6, etc., in table 1. Theoretically the coefficient of variability should be lower if the yield data are combined so that the double sections used for plots in harvesting do not coincide with the areas used as units for filling the beds. To test this conjecture the coefficient of variability was calculated from the double sections 2 and 3, 4 and 5, 6 and 7, etc., as plots. With plots arranged in this way the coefficient of variability drops to 4.1. This corroborates the evidence in the Arlington experiments in favor of arranging the plots so as not to coincide with areas used as units in filling the beds. On an area basis double plots harvested in this manner are 308 percent as efficient as the double plots harvested from the same areas as were used for filling the beds and 334 percent as efficient as the single sections.

DISCUSSION AND CONCLUSIONS

The foregoing experiments demonstrate conclusively that small plots are preferable to entire beds as experimental units in a mushroom house. The variance of the small plots was in all cases less than the variance of entire beds. Furthermore, the small plots permit a greater number of yield comparisons on the same area and also permit increased precision through replication and through the arrangement of the plots so as to reduce the effect of compost heterogeneity and account for the variability between beds.

The results of the uniformity trials at both Arlington and Coatesville indicate an important relationship between the arrangement of the plots and the method employed for filling the beds with compost. When areas were used for harvesting which were comparable in size to the areas used for filling the beds but which overlapped the latter areas there was markedly less variability than when the same areas were used as units for both filling and harvesting. It would seem from this that the customary method of filling the beds by emptying 10 or 12 bushel baskets of compost at a time in each section or by emptying 20 or 24 bushels into each double section induces an excessive variability in the yields from these areas. It is advisable, therefore, to arrange the plots so that they do not coincide with the units of area used for filling the beds. Probably a further gain can be made in the precision of yield tests by modifying the system of filling experimental beds in order to mix the compost and distribute it more uniformly in the beds. A practical method of doing this is to fill the beds by emptying 1 bushel of compost at a time in each section at random in the bed until every section has received the required amount of compost.

This method of mixing the compost in the bed should reduce the variability between plots on a bed, but probably would have little or no effect on the variability between beds. Therefore it is necessary to account for the variability between beds in the reduction of the data. This may be accomplished by randomizing all the treatments on each of the beds so that the beds can be considered as replicate blocks in Fisher's randomized-block system of analyzing the data. As there was no significant fertility gradient (column effect) from one end of the house to the other, randomized blocks are preferable to Latin squares in that they leave more degrees of freedom for error and thus permit a more precise test of significance.

The question of the most desirable size among small plots is somewhat problematic. In all the trials the larger plots varied less than the smaller plots, so that more precision can be expected from the use of whole sections than ½-section plots, double sections than single sections, etc. But in 2 out of the 3 experiments the increase in precision with increase in plot size was not proportional to the increase in plot size. In other words, greater experimental precision was obtained for a given area by increasing the replication of small plots than by increasing the size of the plots. In practice, however, it is usually more expensive to increase replication than to increase plot size; so the experimenter must reach a compromise, depending on circumstances. In the writer's opinion sections 4 by 5 feet make satisfactory plots in a small experimental house such as the one at Arlington Farm and double sections are a good compromise for conventional houses. This allows the comparison of five different varieties or treatments in a single experiment if all the treatments are laid out on each of the replicate beds in accordance with the randomized-block system.

Before leaving the question of plot size, perhaps a word of explanation should be offered for an apparent anomaly in the results, namely, that increased precision is obtained by increasing the size of the small plots on the bed yet when entire beds are used as plots the experiments are less precise. Practical experience suggests that this is due to the fact that greater differences may be expected in the moisture content of the compost, diseases, insect flora, and temperature from the top of the house to the bottom than from end to end. This is substantiated by the greater variation between beds than between columns shown in table 2.

The advisability of replicating treated plots need hardly be discussed in view of the fact, already pointed out, that on an area basis in 2 out of the 3 uniformity trials precision was gained more rapidly through replication than through increase in plot size. The question at issue is how many replications are advisable under the limitations laid down by the conditions of the experiment, the funds available, and the structural features of the experimental house. In the writer's opinion 5 or 6 replications are a good compromise since they will usually enable the investigator to detect differences in yield of approximately 10 percent, due to experimental treatment. If greater precision is necessary it can be obtained through increased replication, as the randomized-block plan allows as many replications as there are beds in the house—usually 12 to 20. When different composts are being compared it is desirable to replicate the compost heaps in addition to replicating the plots derived from each experimental compost heap.

SUMMARY

Yield data are discussed from uniformity trials in the experimental mushroom house at Arlington Experiment Farm, Rosslyn, Va., and in commercial houses at Coatesville and Downingtown, Pa.

These tests indicate that small plots are preferable to beds as experimental units. Double sections containing from 40 to 48 square feet of bed space seemed to be the most practical size of plot for experiments in commercial houses. In one of the experiments there was a distinct advantage in arranging the plots on the beds so as to make it possible to account for the excess variation between beds in analyzing the data.

A gain in precision also was apparent when test plots were so arranged that they did not coincide with the areas used as units for filling the beds. Therefore, a modification of the usual method of filling beds is suggested for experimental yield tests.

At least 5 or 6 replications seem to be necessary.

MAGNESIUM, CALCIUM, AND IRON REQUIREMENTS FOR GROWTH OF AZOTOBACTER IN FREE AND FIXED NITROGEN[1]

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INTRODUCTION

Most of the investigations concerning the inorganic nutrient requirements of *Azotobacter* have not involved comparisons of growth in the presence and absence of fixed nitrogen. The majority have been chiefly qualitative studies in which free nitrogen gas only was used, and they have yielded no evidence as to whether a particular element played a necessary role in fixation or was a general growth nutrient. Burk and Lineweaver (*6*)[2] have recently reported an elaborate series of experiments with both macrocultures and microcultures (Erlenmeyer and Warburg technic) in which calcium (or strontium) was the only metallic ion found to be specifically required in the catalysis of the nitrogen-fixing process at concentrations greater than 0.01 millimolal. Magnesium and iron (*7*) were found to be highly stimulating to the growth process in both free and fixed nitrogen over the respective concentration ranges 0.1 to 3 millimolal and 0.001 to 0.015 millimolal, but no studies were carried out at lower concentrations to ascertain whether these elements were strictly essential.

The present work has extended these investigations in a quantitative manner, with particular reference to the absolute growth requirements. Considerably modified cultural conditions have been employed. The experiments have lasted several days or weeks, instead of 6 to 48 hours, thereby permitting maximum growth. The nutrient media have been maintained at the most recently determined optimum concentrations with respect to all known nutrient requirements except the element or elements under consideration.

REVIEW OF LITERATURE

According to Buchanan and Fulmer (*5*), magnesium is essential to the growth of many, though not all, micro-organisms. Linossier (*22*) has shown that it is required for the development of *Oidium lactis*, Buromsky (*11*) that it is essential for the growth of *Aspergillus niger* and not replaceable by calcium, and Lockemann (*23*) and Frouin and Guillaumie (*13*) that it is not replaceable by calcium, strontium, or any of the rare earth metals in the case of the tuberculosis bacillus. Frouin and Ledebt (*14*) have shown magnesium to be replaceable by certain of the rare earths for the production of pigment by *Bacillus phocaneus*. Many others report stimulating action upon microorganisms at low concentrations of magnesium, with toxicity as the concentration is increased to 100 to 500 millimolal; however, essentialness at very low concentrations has rarely been determined.

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[1] Received for publication Jan. 25, 1934, issued July 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 994.

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Rippel and Stöess (*27*) concluded from experiments with a variety of bacteria and fungi, as well as from a review of the literature, that calcium is not a generally indispensable nutrient for micro-organisms but is occasionally necessary for some special physiological process, such as nitrogen fixation by *Azotobacter*. On the basis of his comprehensive experiments concerning the calcium requirements of living forms, Loew (*24*) concluded that most lower organisms, in contrast to the algae and higher organisms, rarely contain calcium.

Krzemieniewska (*21*), who has made one of the few quantitative studies of the inorganic nutrient requirements of *Azotobacter,* found that 0.16 millimolal of magnesium and 0.12 millimolal of calcium were necessary for maximum growth, whereas zero concentrations of either substantially prevented all growth and nitrogen fixation. The experiments were carried out in free nitrogen only, however, so that a comparison of the action of either element on the growth and fixation processes was not possible. Schröder (*29*) found calcium not to be essential for *Azotobacter* growth in fixed nitrogen, but stimulating, and concluded that this effect was due more to a neutralizing action than to any specific effect of the calcium ion. Stapp and Ruschmann (*30*) found little or no beneficial effect of calcium in a medium containing nitrate.

Very little work has been carried out on the iron requirements for bacterial growth because the limiting concentration range is usually about one-thousandth that of elements such as magnesium or calcium, and most culture media, especially those containing organic nutrients, generally contain sufficient impurity to produce maximum growth. A brief review of the most pertinent previous investigations on general bacterial growth is given by Buchanan and Fulmer (*5, p. 411*). Previous work with respect to *Azotobacter* has been reviewed elsewhere (*7, pp. 441–442*).

METHODS

Several media were employed, termed "basal" (A), "customary" (B), "altered" (C), "humate-free" (D), and "charcoal-treated" (E). The basal medium A consisted of 0.64 g (3.68 millimolal) K_2HPO_4; 0.16 g (1.18 millimolal) KH_2PO_4; 0.2 g (0.81 millimolal) $MgSO_47H_2O$; 0.05 g (0.29 millimolal) $CaSO_42H_2O$; 0.2 g (3.42 millimolal) NaCl; 0.5 mg (0.009 millimolal) Fe as synthetic humate iron[3]; and 20 g (55.5 millimolal) Mallinckrodt's sucrose crystals per liter. This medium was used as a standard control in most of the work. It provided a heavy growth of *Azotobacter* (20 mg organic nitrogen per 100 cc) and did not precipitate upon being sterilized. Customary medium B, employed previously (*6, 7*), contained 1 percent glucose instead of 2 percent sucrose, no humate iron, and slightly more calcium and phosphate. Altered medium C contained more or less added Ca or Mg than basal medium A; it precipitated upon standing if the Mg content were lowered to 0.04 millimolal or the Ca or phosphate content increased 20 percent or more. Humate-free medium D consisted of basal medium A with no Fe added as humate iron. Charcoal-treated medium E was prepared by shaking 1 l of humate-free medium D with 75 g of purified Baker's animal charcoal, allowing to stand for several days, and filtering. Humate iron was added to the filtrate before use in order to insure optimum iron for growth.

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[3] For method of preparation see the following publication: HORNER, C. K., BURK, D., and HOOVER, S. R THE PREPARATION OF HUMATE IRON AND OTHER HUMATE METALS. Plant Physiol. (In press.) 1934.

Preliminary purification of the charcoal in order to remove considerable amounts of adsorbed gases and other impurities was accomplished by leaching 3 to 6 times at intervals of several hours with equal weights of 6 N HCl and finally washing on a Buchner filter with dilute alkali and then distilled water until the wash water was neutral.

Fixed nitrogen when employed was added at the rate of 100 to 200 mg (7.1 to 14.2 millimolal) per liter, as KNO_3, NH_4Cl, or urea.

Erlenmeyer or Florence flasks of 150 to 250 cc capacity containing 15 to 25 cc of nutrient solution and plugged with cotton were employed as culture flasks. Sterilization was accomplished by autoclaving for 10 minutes at 15 pounds pressure, 20 percent extra distilled water being added to each flask to make up for loss by evaporation. The culture flasks were inoculated with 3 drops of culture grown in aeration bottles containing 100 cc of customary medium. In the experiments where it was desired to minimize as much as possible any Ca or Mg carried over by the inoculum, heavy 2- to 7-day cultures were diluted 50 to 100 times with sterile medium free from these elements. The cultures were incubated in duplicate or triplicate at 28° to 30° C. for 2 to 10 days, occasionally longer, with frequent observations.

Growth was measured turbidimetrically with a Bausch & Lomb nephelometer, corrections being made for any significant original turbidity in uninoculated cultures (cultures of low Mg content). The original and final pH values were measured colorimetrically by using the various La Motte indicators and standards. The original pH of all media was 7.0 ± 0.2. In general, the pH values tended to decrease a maximum of 1 to 2 units with increase in growth, except in the case of cultures with nitrate, where the values often increased. The buffering capacity of the medium was substantially independent of the Ca or Mg concentration, being controlled chiefly by the phosphate concentration. In general, the sugar was rarely entirely consumed by the end of an experiment.

The organism employed was *Azotobacter vinelandii*, capable of very active nitrogen fixation and long in use at this laboratory. According to the recent work of Kluyver and van Reenen (*18*), this species is the common motile form of *Azotobacter*, of relatively small size, occurring in soils. It produces a green fluorescent pigment, and at times assumes either a lighter, yellow, or darker, pink or purple, tinge, especially in the presence of molybdenum.

EXPERIMENTAL RESULTS

MAGNESIUM AND CALCIUM

Tables 1, 2, and 3 show fair examples of the relative growths obtained with *Azotobacter* with a wide variety of Mg and Ca concentrations under otherwise approximately optimal conditions. The ratios of growths in altered media C to those in basal medium A are given as the most direct means of comparing the results in the different experiments at various stages of growth and with the three forms of nitrogen employed. Table 1 shows the effect upon growth of lowering either Mg or Ca while the other element is kept constant at the usual concentration; the results with varying Mg in the 10-day experiment are plotted in figure 1.

In general, the fraction of normal growth at any concentration of Mg is roughly the same in free or fixed nitrogen, although the actual growth may be 2 to 4 times as great in the latter. The fraction tends to become smaller with the duration of the experiment and extent of growth, as would be expected if Mg were an essential cell nutrient. In other experiments lasting 30 to 40 days, cultures without Mg were only about one-hundredth as turbid as the basal medium controls.

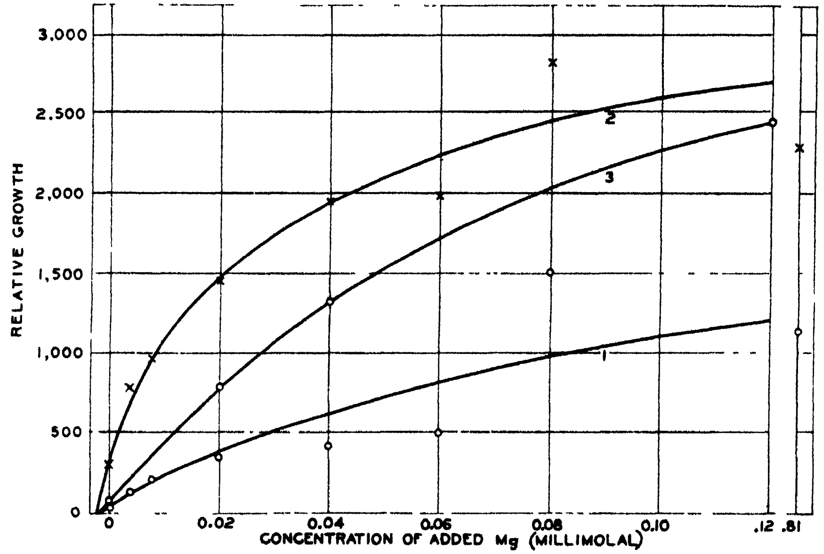

FIGURE 1.—Growth of *Azotobacter* in free and fixed nitrogen as a function of magnesium concentration. Curves 1 and 2, growth (turbidity) in N_2 and nitrate, respectively, 10-day experiment (table 1). Curve 3, growth (nitrogen fixed) in N_2, 12-day experiment, data of Krzemieniewska (*21*) (ordinate scale different from that for curves 1 and 2).

The growth of *Azotobacter*, independently of the source of nitrogen, decreases from normal at 0.05 to 0.1 millimolal Mg to one-fiftieth to one-hundredth of normal at 0.002 millimolal Mg (estimated maximum impurity in basal medium and inoculum).

TABLE 1.—*Effect of Ca and Mg concentrations upon Azotobacter growth and fixation*

Ca	Mg	Growth (turbidity) ratio,[a] altered medium C / basal medium A in—					
		Experiment 1, 3 days			Experiment 2, 10 days		
		N_2 (air)	KNO_3	Urea	N_2 (air)	KNO_3	Urea
Millimolal	*Millimolal*						
[b] 0.29	[b] 0.810	1.000	1.000	1.000	1.000	1.000	1.000
.29	.081	1.000	.573	.940	1.320	1.240	1.300
.29	.061	.356	.382	.658	.450	.873	1.270
.29	.040	.339	.339	.439	.367	.855	.795
.29	.020	.339	.329	.387	.306	.640	.306
.29	.008	.319	.329	.327	.181	.423	.183
.29	.004	.319	.267	.204	.122	.350	.144
.29	.000	.257	.114	.084	.037	.141	.066
.15	.810	.808	.958	.800	.718	.953	1.120
.06	.810	.628	1.040	.648	.768	1.050	1.220
.03	.810	.532	.840	.550	.568	1.295	1.190
.006	.810	.172	.710	.523	.437	.940	1.060
.00	.810	.130	.545	.470	.295	.606	1.190

[a] Relative growths in basal medium A—N_2: NO_3:urea (3 days) = 1:3.7:3.7; (10 days) = 1:2.0:4.1.
[b] Basal medium A.

TABLE 2.—*Effect of variable Ca-Mg ratios upon Azotobacter growth and fixation*

Ca	Mg	Ca/Mg	Growth (turbidity) ratio,[a] $\frac{\text{altered medium C}}{\text{basal medium A}}$ in								
			Experiment 1, 4 days			Experiment 2, 7 days			Experiment 3, 10 days		
			N_2 (air)	KNO_3	Urea	N_2 (air)	KNO_3	Urea	N_2 (air)	KNO_3	Urea
Milli-molal	*Milli-molal*										
0.290	0.0	∞	0 162	0 628	0 230	0 101	0 315	0 093	0 032	0 332	0 073
.029	0	∞	085	424	280				069	.202	085
012	0	∞				.016	175	074			
.290	.081	3 58	1 220	.695	1 565				981	.935	1 010
012	016	.75				.015	589	448			
006	008	75				018	438	214			
[b] 290	[b] 810	36	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
087	240	36	918	.742	1 190				.705	565	1 000
.029	081	.36	166	.935	878				158	935	.885
[c] 004	[c] .028	14	130	653	638	.009	667	1 000	016	.556	1 030
.029	81	036	695	910	878				.276	764	788
029	2 43	012	855	1 125	553				329	658	963
.029	4 86	.006	618	1 125	807				287	633	1 030
0	4 86	0	.297	963	745				158	.568	980
0	2 43	0	364	1 030	1 090				333	475	878
0	81	0	173	910	1.150	164	642	972	142	.424	1 050
0	.081	0	067	963	.807				.037	742	953
.0	016	0				020	547	396			
0	0		068	454	214	.013	317	108	010	263	089

[a] Relative growths in basal medium A - N_2 NO_3 urea (4 days) = 1 0 2 7 4 8, (7 days) = 1 0 1 4 2 1, (10 days) = 1 0 0 9 1 5

[b] Basal medium A

[c] Charcoal-treated medium E

TABLE 3.—*Influence of Ca in medium and inoculum upon growth in free and fixed nitrogen*

Nitrogen source	Ca in medium	Ca in inoculum	Growth (turbidity) in		
			2 days	3 days	6 days
N_2	[a] +	[a] +		37	123
	+	−		36	107
	[b] −	[b] +		7	24
	−	−		6	26
KNO_3	+	+	58	121	234
	+	−	56	92	227
	−	+	25	65	286
	−	−	25	75	149
Urea	+	+	41	212	484
	+	−	34	172	374
	−	+	43	209	472
	−	−	29	121	455
NH_4Cl	+	+	88	166	231
	+	−	52	141	231
	−	+	81	83	123
	−	−	60	100	140
Average of KNO_3, urea, and NH_4Cl	+	+	63	167	316
	+	−	47	135	277
	−	+	50	179	293
	−	−	38	99	248

[a] Basal medium A.

[b] Altered medium C (basal medium A without Ca). KNO_3 present in Ca-free medium used to prepare inoculum; such inoculum grown for 3 transfers previous to use

When the Ca of the medium is lowered, a different relationship is seen to exist. Whereas the growth in fixed nitrogen may be decreased a maximum of 50 percent, that in free nitrogen is lowered 70 to 90 percent when but traces of Ca are present. Ca deficiency is more pronounced in the early stages of growth in both free and fixed nitrogen. As growth progresses the need for Ca in fixed nitrogen is practically nullified; and, whereas a definite effect remains in free nitrogen, after a sufficient time (30 to 40 days) mere traces of Ca will permit 25 to 50 percent of normal growth. This substantiates the conception that Ca acts in a truly catalytic role in nitrogen fixation; only traces are absolutely necessary provided sufficient time is allowed, but relatively high concentrations are required to produce a maximum rate of fixation. The Ca needed for normal maximum growth in free nitrogen is 0.15 to 0.30 millimolal or practically saturation (0.36 millimolal), and this range appears to hold also in the early stages of growth in fixed nitrogen, but decreases after several days to less than 0.01 millimolal in nitrate and presumably to zero in urea (and ammonia).

The decrease with the duration of the experiment in the Ca requirements for growth in fixed nitrogen is indicated in table 3. The culturing of the inoculum for several previous transfers in Ca-free medium does not enable the organisms to become adapted to such a medium since somewhat less growth is obtained, as compared to inoculum grown in a medium containing calcium. This is best seen in the "average" values and tends to indicate a very small but definite Ca requirement in fixed nitrogen for entirely maximum, as distinguished from approximately maximum, growth. It is seen that the Ca requirement in free nitrogen remains considerable even after 6 days, the ratio of growth in its presence and absence (except for impurities) being 6 to 1 at 3 days and 4½ to 1 at 6 days.

In table 2 are given the growth ratios with a variety of Ca/Mg ratios principally under suboptimal concentrations of both elements. The most definite results are obtained with the older cultures. It is seen that little if any effect can be attributed to the Ca/Mg ratio, as has been reported frequently with supraoptimal concentrations with various organisms in connection with antagonism (*5, pp. 282–284, 356–359*). The independent effect of each element can be analyzed. Varying the Ca in the absence of Mg has very little effect in any case, since the lack of Mg is already limiting the growth almost completely. The two 0.75 Ca/Mg ratios show no significant difference in N_2, the Ca and Mg both being highly limiting; in fixed nitrogen the decrease corresponds to the Mg decrease. The three 0.36 ratios, with widely varying concentrations of the two elements, show definite decreases of growth in N_2 (due to calcium deficiency), but relatively little in fixed nitrogen. Keeping the Ca constant at 0 or 0.029 millimolal and increasing the Mg sixfold from 0.81 to 4.86 millimolal causes no consistent significant change in the amount of growth. As indicated previously, toxic effects are obtained at concentrations much greater than 4.86 millimolal (6 "customary").

The charcoal treatment of basal medium A was developed to remove, if possible, some nutrient or nutrients specifically required in growth or fixation, either in large or very small concentration. Hopkins (*17*) used this method (without the charcoal purification) to remove traces of iron from dissolved sugar. As shown in table 2 (Ca/Mg = 0.14), the effect of the treatment can be attributed almost entirely to the Ca and Mg removed; the Ca was found upon analysis, by an oxalate precipitation method, to have been reduced to about one-seventieth, and the Mg, by an ammonium phosphate precipitation method, to one-thirtieth of the original concentrations. The Mg retained is still sufficient to permit 50 to 100 percent normal growth in fixed nitrogen, whereas in free nitrogen the combined Mg-Ca deficiency permits but slight growth, owing chiefly to lack of Ca and secondarily to lack of Mg; the effect of Mg deficiency is enhanced at low Ca. The average growth ratios in the charcoal-treated medium E for seven different 6- to 10-day experiments were: N_2, 0.015; KNO_3, 0.595; urea, 0.715. As may be seen in table 2, these growth ratios are approximately those obtained when the same order of Ca and Mg concentrations are added intentionally in making up altered media.

The preponderant influence of Ca-Mg deficiency in the charcoal-treated medium E was also demonstrated by determining the effect of adding thereto the different basal medium A salts separately. The KH_2PO_4, K_2HPO_4, and NaCl caused substantially no improvement. Humate iron was normally added to all charcoal-treated medium E, but special experiments involving its omission showed that it likewise was not beneficial. Table 4 shows the effect of adding Mg and Ca separately and together to the charcoal-treated medium E. The most striking results, as would be expected, are observed in free nitrogen. Ca alone increases the growth about 7 times, Mg about 18 times, and both together about 45 times, or to within 25 percent of that in basal medium A. The fact that Ca and Mg together did not cause complete recovery indicates that some other slight inhibiting factor may be involved in the charcoal treatment, but a 10- to 30-percent variation can be expected in the methods of culturing and measurement employed. Since the deficiency in the case of fixed nitrogen is not sufficient to cause more than this amount of inhibition, no definite beneficial effect of the added Ca and Mg can be detected. Neither the Ca nor the Mg alone is sufficient to return the growth to normal since the other element is always deficient. When either Mg or Ca solutions (of basal medium A concentration) are treated with charcoal and added to the other salts untreated, both media so obtained show growth inhibition, as would be expected. This inhibition is overcome by adding Mg or Ca, respectively. Similar treatment of phosphate or NaCl solutions yields no inhibition. It may be stated also that the extent of reduced growth obtained in charcoal-treated medium E was independent of the concentration of Fe added and also the amount or age of inoculum. Finally, sugar charcoal as compared with animal charcoal yielded no growth reduction; in fact it was observed to provide iron in media deficient in this element.

TABLE 4.—*Growth recovery in charcoal-treated medium E upon addition of Mg or Ca*

Medium	Growth (turbidity) ratio,[a] $\frac{\text{altered medium C}}{\text{basal medium A}}$ when source of nitrogen was—		
	N_2 (air)	KNO_3	Urea
Basal A	1.000	1.000	1.000
Charcoal-treated E	.016	.787	.708
Charcoal-treated E+0.29 millimolal Ca	.115	.805	[b].565
Charcoal-treated E+0.81 millimolal Mg	.297	[b].595	.815
Charcoal-treated E+0.29 millimolal Ca and 0.81 millimolal Mg	.732	.787	.808

[a] Relative growths in basal medium A—N_2:NO_3:urea (6 days)=1.0:1.0:1.7.

[b] Low.

The apparent essentialness of Mg for *Azotobacter* growth, as observed in the preceding experiments, is almost certainly a true magnesium requirement. The Baker's $MgSO_4.7H_2O$ employed was examined spectroscopically.[4] Only sodium was found in considerable amount, with definite but faint indication of aluminum, copper, calcium, iron, lithium, and silicon, and possibly vanadium and boron (no potassium). All these elements, and others, were tested for their effect in either charcoal-treated medium E or basal medium A containing no Mg. The following metal ions were added to charcoal-treated medium E: Cu, Mn, Ni, Co, Al, Zn at 0.1 p.p.m.; Mo, Si, Ti, Cr at 1 p.p.m.; V, Mn, B, Li, and Ba at 0.1 and 1 p.p.m.; 20 and 200 p.p.m. of natural humic acid, a material which undoubtedly contains traces of all basic elements normally found in soil; and also 10 p.p.m. coenzyme R (*1*). No increase in growth was observed with any of these. In the magnesium-free medium the following metals were tested: V, B, Mo, and Li at 2.5 and 10 p.p.m.; Ba and Sr at 2.5, 10, 25, and 50 p.p.m.; and Mn at 0.5, 2.5, 10, and 12.5 p.p.m. Mn, Ba, and Sr appeared to be slightly beneficial. The other elements yielded no increase in growth. Mg as such is therefore essential with no element able to act very efficiently in place of it. No stimulation in basal medium A resulted from 0.1 and 1 p.p.m. Cu, Al, Ni, and Mn, but stimulation was often increased 1½ to 3 times by either V (VCl_3, Na_3VO_4, or $K_2V_4O_9$) or Mo ($NaMoO_42H_2O$) at 1 p.p.m., in agreement with previous findings of Bortels (*3*, *4*), Burk and Lineweaver (*6*), Birch-Hirschfeld (*2*), Schröder (*29*), Kluyver and Van Reenen (*18*), and Konishi and Tsuge (*19*).

IRON

The problem of demonstrating a definite iron requirement usually necessitates purification of the medium rather than the addition of iron, owing to the low values involved. The phosphate-adsorption method reported by Hopkins (*17*) in connection with the green alga *Chlorella*, and the carbonate treatment described by Steinberg (*31*) in connection with the bread mold *Aspergillus*, for removing traces of Fe were found to be incapable of causing any Fe deficiency for *Azotobacter* in basal medium A without humate iron (medium D). There still remained after treatment 0.001 to 0.003 millimolal Fe. The modified Hopkins' charcoal treatment employed for removal of Mg and Ca masked any possible removal of Fe. Ruhland's (*28*) method of autoclaving and filtering a nutrient solution composed only of inorganic nutrients was found to lower the Fe impurity of the inorganic salts of basal medium A but could not be employed for sugar purification. Several sugars were, therefore, obtained, which when analyzed by a method previously described (*7*, *table 1*), showed a varying and relatively low Fe content, as follows: Difco dextrose, 4×10^{-4} percent; Merck's dextrose, 6×10^{-5} percent; Baker's sucrose, 6×10^{-5} percent; and Mallinckrodt's sucrose, 9×10^{-6} percent. The inorganic basal medium A (without humate iron or sugar) contained before and after autoclaving and filtering while hot 2.6×10^{-5} millimolal and 1.7×10^{-5} millimolal Fe, respectively. It was possible to obtain 8 Fe concentrations ranging from 5×10^{-6} millimolal to 4.5×10^{-4} millimolal by employing each of the 4 sugars at 13.9 millimolal (0.5 percent) together with 41.6 millimolal (1.5 percent) of the

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[4] These examinations were made by the Bausch & Lomb Optical Co.

Mallinckrodt's (very low iron) sucrose, basal inorganic medium A being used with and without $5 \times 10^{-[illegible]}$ millimolal humate iron (Fe impurity in synthetic humic acid with no Fe intentionally added); the lowest concentration was obtained by using 55.5 millimolal (2 percent) Mallinckrodt's sucrose in inorganic medium previously autoclaved and filtered. Maximum iron was assured by adding 1.8×10^{-3} millimolal humate iron to some of the cultures.

Figure 2 shows the relative growths obtained with these varying Fe concentrations. The curves definitely approach zero growth at the lowest concentrations, whereas the sugar, Difco dextrose, with the maximum Fe impurity, 4×10^{-5} millimolal, yields practically normal growth. The concentration of Fe yielding maximum growth increases somewhat with age of culture (curves 1 and 1*a*, 2 and 2*a*), as was shown previously in a different manner in connection with humic acid stimulation (*8, p. 458, fig. 2*). At any given age of culture approximately the same differences in amount of growth in free and fixed nitrogen are obtained at all iron concentrations (table 5), indicating that, as determined by this method, there was no specific fixation effect of Fe deficiency, such as was obtained so definitely in the case of Ca. The phenomenon of the growth in free nitrogen approaching that in fixed nitrogen, after a prolonged period of time, is shown by the "average" values, table 5, and by comparing the ordinate ranges between curves 1 and 2 and curves 1*a* and 2*a*. This phenomenon is commonly observed and presumably bears no direct relation to Fe requirements, but to limitations by other factors (*8, pp. 455–462*). Ruhland (*28*) has reported a limiting Fe concentration range for the autotrophic hydrogen organism *Bacillus pycnoticus* which is essentially the same as that demonstrated for *Azotobacter*, no growth being obtained at 10^{-5} millimolal and maximum growth occurring by 1.2×10^{-3} millimolal.

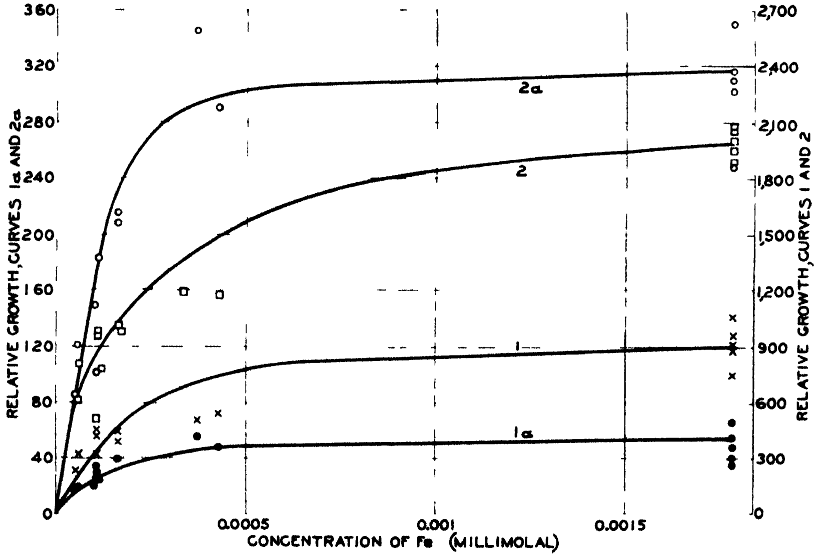

FIGURE 2.—Growth of *Azotobacter* in free and fixed nitrogen as a function of iron concentration. Curves 1 and 2, growth (turbidity) in N_2 and nitrate, respectively, 9-day experiment; curves 1*a* and 2*a*, 2-day experiment.

TABLE 5.—*Ratio of growth in fixed and free nitrogen at different iron concentrations*

Concentration of Fe (millimolal)	Growth (turbidity) ratio, $\frac{KNO_3}{N_2}$ for experiment—			
	No 1[a]		No 2	
	2 days	9 days	3 days	5 days
4.8×10^{-5}	5.0	2.7		
5.9×10^{-5}	6.7	2.6		
1.01×10^{-4}	5.5 {3.1 6.0 7.0	2.3 {2.3 2.2 2.4		
1.1×10^{-4}	7.0	2.4		
1.6×10^{-4}	5.6 {5.4 5.7	2.4 {2.3 2.5		
4.1×10^{-4}	6.2	2.3		
4.6×10^{-4}	6.1	2.2		
1.8×10^{-3}	6.6 {5.4 8.0 3.8 7.3 8.7	2.2 {2.0 2.1 2.0 2.3 2.6		
1.8×10^{-2}	5.7 {5.8 7.4 2.7 5.6 7.1	2.1 {1.6 2.0 2.6 1.9 2.4		
2.5×10^{-5}			4.6	4.3
3.6×10^{-5}			3.0	3.5
8.1×10^{-5}			5.7	2.6
3.8×10^{-4}			4.3	3.3
1.8×10^{-2}			4.5 {4.3 4.3 5.3 4.2	3.1 {3.1 2.9 2.0 4.4
Average	6.1	2.3	4.4	3.4

[a] Compare fig 2, p 989

COMPARISON OF ABSOLUTE ELEMENT REQUIREMENTS

Comparing the concentrations of essential elements producing half maximum growth ($C_{G/2}$) is as convenient, and more accurate, than comparing those required for optimum growth, under variable experimental conditions. Table 6 gives $C_{G/2}$ values for Ca, Mg, and Fe under various conditions of age of culture and nitrogen source, and also those for Ca and Mg determined from Krzemieniewska's data (*21*). These values have been determined graphically from curves such as those shown in figure 1 for Mg and in figure 2 for Fe. The values for Mg and Fe do not vary significantly with respect to nitrogen source, being essential elements in any case, whereas the nonessentialness of Ca in fixed nitrogen is strikingly shown by the lack of any definite positive value, as compared with 2×10^{-2} to 5×10^{-2} millimolal in free nitrogen. $C_{G/2}$ for Fe is about one five-hundredth of that for Mg or Ca in free nitrogen.

In general, $C_{G/2}$ should decrease with age or heaviness of cultures if the element is utilized relatively more efficiently at lower concentrations by being employed over and over again in a catalytic manner. It should increase if the element is consumed and prevented from further functioning. As can be seen from table 6, there are no changes in $C_{G/2}$, for any of the elements, great enough to distinguish conclusively between catalysis or consumption or a combination of both. A tendency appears for a decrease with Mg and Ca and an increase with Fe, and earlier evidence with Ca and Fe supports this interpretation. $C_{G/2}$ probably varies relatively little for growths ranging from 2 to 20 mg organic nitrogen per 100 cc (light to very heavy growths, about 20 to 200 mg dry matter per 100 cc); it should be a fairly characteristic constant. This is important in connection with the basal medium A employed, since normally Mo and V were not added, although in experiments involving very heavy growth they could cause definite increases in growth.

TABLE 6.—*Approximate concentrations of calcium, magnesium, and iron required for half maximum growth of Azotobacter using various sources of nitrogen*

Element	$C_{G/2}$, the concentration (millimolal) yielding half-maximum growth when source of nitrogen was							
	N_2 (air)		KNO_3		Urea		N_2 (Krzemieniewska (*21*))	
	2-3 days	9-10 days	2-3 days	9-10 days	2-3 days	9-10 days	5 days	12-15 days
Ca	4×10^{-2}	2×10^{-2}	[a]$0-2\times10^{-4}$	[a]$0-2\times10^{-4}$	[a]$0-2\times10^{-4}$	[a]$0-2\times10^{-4}$	8×10^{-2}	5×10^{-2}
Mg	6×10^{-2}	4×10^{-2}	6×10^{-2}	2×10^{-2}	5×10^{-2}	3×10^{-2}	5×10^{-2}	4×10^{-2}
Fe	1.1×10^{-4}	1.4×10^{-4}	1.1×10^{-4}	1.6×10^{-4}	---	---		--

[a] Maximum Ca impurity in medium and inoculum.

Krzemieniewska (*21*) reported that potassium, sulphur, and phosphorus were required for *Azotobacter* growth in relatively large concentrations, the first-named at about that found for Ca and Mg, and the latter at a value 3 to 10 times as great. Vogel (*32*) and Stapp and Ruschmann (*30*) report, however, that the addition of potassium to media was not required. Similarly the present writers, and Stapp and Ruschmann (*30*), have found that the traces of sulphur occurring as impurities will yield at least 50 to 75 percent of maximum growth. The phosphorus requirements have been generally assumed to be relatively high; Burk and Lineweaver (*6*) have indicated that 0.1 millimolal is sufficient.

DISCUSSION

The experiments of long duration reported above are seen to confirm the previous observations (*6*) involving experiments essentially of short duration concerning the catalytic effect of Ca upon the nitrogen-fixation process of *Azotobacter*. The small concentrations of Ca studied in the present work yield still more evidence that Ca acts in a truly catalytic role. The relatively large concentrations emphasized previously (0.10 to 0.30 millimolal) applied to obtaining the maximum rate of fixation by young cultures, rather than to the maximum amount of growth after a long period of time. The apparent stimulation of growth in fixed nitrogen by Ca is much lessened, if not upon occasion entirely eliminated, as the duration of the experiment is increased.

The essentialness of Mg for the growth of *Azotobacter* is much more evident from the experiments of long duration, with the other inorganic nutrients at normal concentrations, than in the experiments of Burk and Lineweaver (*6*) where 10 times the customary diluted medium was chiefly employed. Whereas some growth may occur in free nitrogen with less than 2×10^{-3} millimolal Mg (estimated maximum Mg impurity) in the first 24 to 48 hours, surpassing that obtained with a medium containing 0.81 millimolal Mg, but no Ca, growth in the former practically ceases after a few days, whereas in the latter a definite increase occurs. In this case the Ca is being used continuously at very small concentrations.

As mentioned above, and observed in a former paper (*6, p. 164*, "pseudo-Ca-Sr-effect"), Mg appears to possess at times a somewhat, greater relative beneficial effect upon growth in free nitrogen than in fixed nitrogen, particularly at low concentrations of Ca. For the present, this is still interpreted as involving no specific influence upon the fixation process proper, but probably making any Ca present in the cells more available, perhaps merely more soluble. In this connection, Mg has been observed to interfere markedly with Ca precipitation by oxalate, and also, as mentioned above, serves to maintain a clear nutrient solution by increasing the solubility of calcium phosphate. This effect upon Ca solubility would scarcely be related to the chief effect of Mg in the growth process, since Ca is substantially unnecessary there.

Haines (*16*) has reported a somewhat parallel relationship with Ca and Mg between the production of bacterial gelatinase and growth with five different micro-organisms. Ca alone promotes the formation of the enzyme while permitting but poor growth; Mg alone yields good growth but substantially no gelatinase. The best effect is obtained in the presence of both elements, probably owing in part to the influence of growth itself upon the protease production. In a previous work, Haines (*15*) reported a similar effect with the enzyme attacking caseinogen. He suggests that future work might show salts of Ca to be essential for the formation of proteases in general. Although this view might appear contradictory to Rippel and Stoess' (*27*) conclusion as to the general unessentialness for Ca for micro-organisms, since the majority probably produce proteases, it suggests in connection with the present work with *Azotobacter* that Ca when essential for organisms is a constituent of some particular enzyme. Thus, Nakamura (*26*) has found that Ca is an essential activator for amylase. The concentration of Ca has recently been shown, however, to have no influence on two specific aspects of fixation by *Azotobacter*, namely, the pH limit at 6, and the Michaelis dissociation constant or nitrogen pressure at which half maximum velocity of fixation is obtained (*9*).

Mg may also act specifically as a constituent of certain frequently occurring enzymes, as well as in some general phenomenon such as permeability. Lohmann (*25*) has found that Mg is an essential constituent of the coenzyme for lactic acid formation in muscle and for cozymase in yeast, and Erdtman (*12*) has found that it is necessary for liver phosphatase. The general utilization of Mg in oxidative processes is improbable, however, because of their diversity in the various organisms. Kruse, Orent, and McCollum (*20*) have shown that Mg deficiency in young rats leads to death and that the most striking blood change is a disturbance of the lipoid distribution. This finding may disclose upon further investigation that Mg is concerned generally in some phase of lipoid behavior.

The observation that the iron requirement of *Azotobacter*, as measured by final amounts of growth obtained with the Erlenmeyer technic, is substantially independent of the source of nitrogen confirms the previous finding (*8, pp. 465, 482, 486; 10, p. 523*) that in experiments of this type the stimulation caused by iron supplied to customary medium, as humate or otherwise, is approximately the same in free and fixed nitrogen: that is, iron does not normally appear to influence directly or specifically the availability of either of these sources of nitrogen, the one more than the other. The very much lower concentrations of Fe as compared to Ca required in free nitrogen make a specific fixation requirement somewhat more difficult to determine by the methods here employed. In earlier papers reference was made (*7, pp. 427, 447; 8, p. 465 and table 6; 10, p. 523*) to unpublished work on the apparent specific effect of iron in fixation under certain particular conditions, obtained with the Warburg technic, where the velocity constants as distinguished from the final amounts of growth were observed. This apparent specific effect of iron has now been definitely determined to be due to an exceedingly small molybdenum impurity in the iron. The molybdenum is effective at a concentration of only 1 to 100 mg[4] per 1,000,000,000,000 mg medium (1 to 100 parts per trillion, or 10^{-11} to 10^{-9} molal Mo.)

The amounts of iron required for maximum growth of *Azotobacter* as determined in the experiments reported here are approximately the same as those indicated in previous work (7), namely, 0.0004 to 0.01 millimolal (0.02–0.5 p.p.m.). In the experiments described in table 5 and figure 2, the concentration 0.018 millimolal as compared to 0.0018 millimolal gave growths 10 and 22 percent higher, on an average, at 2 and 9 days, respectively. No similar comparison of $C_{G/2}$ values is possible, since it is only in the present work that the low concentrations of Fe have been studied.

SUMMARY

The magnesium, calcium, and iron requirements for growth of *Azotobacter vinelandii* in the presence of free and various forms of fixed nitrogen have been investigated quantitatively, and brief reference made to other inorganic elements. Various types of media were employed. In comparison with work reported previously, the experiments were of relatively long duration (2 to 10 days), and the growths obtained were very heavy (20 to 200 mg dry matter per 100 cc).

The concentrations of the respective elements yielding half maximum growth ($C_{G/2}$) have been determined as the most accurate means of making various comparisons. In these experiments $C_{G/2}$ was relatively independent of duration of experiment and extent of growth. $C_{G/2}$ for Ca is $2\text{–}5 \times 10^{-2}$ millimolal in free nitrogen, and negligible ($0\text{–}2\times10^{-4}$ millimolal) in fixed nitrogen, such as nitrate, ammonia, or urea; this difference confirms previous findings concerning the specific role of calcium in the nitrogen-fixing process. With Mg and Fe, $C_{G/2}$ is independent of the source of nitrogen, being $2\text{–}6 \times 10^{-2}$ and $1.1\text{–}1.6\times10^{-4}$ millimolal, respectively. The essential role of both Mg and Fe in growth is indicated by an approach to zero growth at the lowest concentrations. No specific requirement of Fe (or Mg) in fixation was evident in experiments of the type here employed, confirming previous findings that normally humate iron exerts the same stimulation in free and fixed nitrogen.

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The concentrations of Mg, Ca (in free nitrogen), and Fe required for maximum growth are 0.05–0.1 millimolal, 0.1–0.3 millimolal, and 0.0004–0.001 millimolal, respectively. The requirement for P appears to be 0.1 millimolal, and for S, K, Mo, and V equal to or less than that for Fe.

Mg could not be replaced in the growth process by Cu, Mn, Ni, Co, Al, Zn, Ca, Sr, Ba, Mo, Si, Ti, Cr, V, B, or Li applied in various concentrations.

In ascertaining the very low Fe requirement, adsorption methods involving charcoal, calcium carbonate, or calcium phosphate did not suffice to free the medium from iron. It was necessary to select sugars with different and very low amounts of iron. This method should be useful in connection with various elements similarly needed in traces in general bacterial growth.

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THE NORMAL DEVELOPMENT OF THE LEG BONES OF CHICKENS WITH RESPECT TO THEIR ASH CONTENT[1]

By H. M. Harshaw, *assistant biochemist*, J. C. Fritz, *junior biologist*, and Harry W. Titus, *biological chemist*, *Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The ash content of certain bones, especially those of the legs, has been used in recent years as a measure of the effect of experimental diets on the mineralization of the skeleton of the animals to which the diets were fed. This has been particularly true in some of the nutrition studies with chickens, in which the percentage of ash in one or another of the leg bones has been used to determine the extent of the occurrence of rickets. The ease with which rickets may be produced in chickens has led to the use of chickens as laboratory animals for determining the antirachitic value of the various vitamin D supplements, particularly cod-liver oil, used in diets for poultry.

In order that a correct interpretation may be placed on the results of work of this nature, it is necessary that data on the ash content of the bones of birds reared under normal environmental conditions be available for the purpose of comparison. Furthermore, these data should furnish information on the effect, if any, of age, breed, and sex on the ash content of the bones. The published percentages of ash which have been considered as normal were obtained almost wholly from the analysis of the bones of birds reared under rather artificial conditions, and they represent only one or, at best, a few ages. It is necessary also to know how the several factors other than diet affect the deposition of the mineral elements in bone, for otherwise one cannot be certain that the results obtained are due only to the diets, or, more specifically, to the vitamin D supplements being studied.

Furthermore, it is often desirable to compare the results of work carried out in different laboratories, and in order that this may be possible it is necessary that comparable methods be used for the determination of the ash in the bones of the experimental birds. At present there does not seem to be any great degree of unanimity among the various investigators in this respect. In general the tibia has been used for this purpose, but in some cases other bones have been used. Some reports show different ways of preparing the bones for analysis; others show the use of different methods for ash determination. Some of these differences probably had little or no effect on the results obtained, but it is also probably true that some had a marked effect.

This lack of uniformity in the methods used for the determination of the ash of the leg bones, as well as the lack of adequate information regarding the percentage of ash in the bones of chickens reared under normal conditions, made it desirable to ascertain the ash content of the bones of such chickens of both sexes and of different ages and breeds by the use of a simple and rapid method.

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PLAN OF THE INVESTIGATION

It was planned to rear two groups of chickens, each group of a different breed, under favorable conditions on a grass range, and to study the normal development of their leg bones with respect to the ash content. Each week, for 20 weeks, representative birds were to be selected from each group to furnish the bones to be studied. The plan involved the determination of the ash content of each of the three major long bones of the right leg and of the combined bones of the left leg (1) with the epiphyseal cartilages retained and (2) with the epiphyseal cartilages removed.

To obtain supplementary information as a check on the ash content of the bones, it was planned to determine the calcium and phosphorus content of the blood serum and of the ash of the tibiae, and to note, by means of X-ray shadowgraphs, the age at which calcification occurred at the distal and proximal ends of the tibiae and the proximal end of the metatarsus. The blood-serum studies were conducted for 25 weeks; the other studies for 20 weeks.

EXPERIMENTAL MATERIAL AND METHODS

Three hundred and fifty chicks of each of the two breeds used (Rhode Island Red and Single-Comb White Leghorn) were hatched on April 8, 1931, at the United States Animal Husbandry Experiment Farm, Beltsville, Md., and were placed, the next day, in colony brooder houses on a grass range. At that time 100 chicks of each breed were selected at random and wing-banded. These birds were weighed then and at intervals of 2 weeks thereafter for 20 weeks, in order to obtain a reliable indication as to whether the average growth of the birds was satisfactory.

For the first 14 weeks, the birds were fed the following dry mash:

	Percent
Yellow corn meal	41. 50
Ground wheat	20. 00
Wheat bran	15. 00
Dried buttermilk	10. 00
Meat scrap (50 percent)	10. 00
Calcite	2. 75
Salt	. 75
Total	100. 00

In addition to this mash, during the first 6 weeks after hatching the birds were given all the freshly soured skim milk they would consume. After the fourteenth week and until the end of the twentieth week, all except 2.5 percent each of the meat scrap and dried buttermilk was replaced by yellow corn meal.

On the day after the chicks were hatched and at intervals of 1 week thereafter, a sufficient number of individuals of each breed was selected at random from among the unbanded birds to furnish the leg bones necessary for the ash determinations. The number for each sample varied from 1 to 15, depending on the size of the chicks.

Calcium and inorganic phosphorus determinations were made on the blood serum of the birds selected, in order that it might be known whether they were normal in this respect. Blood samples of approximately equal size were obtained by heart puncture from each of these birds before they were killed for bone samples. The blood samples of birds of the same sex and breed were then combined for analysis. It was not possible, however, to differentiate readily between the sexes until the age of 6 weeks in the case of the Leghorns, and until the age of 7 weeks in the case of the Rhode Island Reds. Accordingly, until the sex of the chicks could be determined, the blood samples were combined with regard to breed only. The serum, obtained by centrifuging the blood samples, was analyzed for calcium by the Clark and Collip (*2*) [2] modification of the method of Kramer and Tisdall; and for inorganic phosphorus, by the method of Fiske and Subbarow (*3*).

After the collection of the blood samples, the birds were killed by cutting the jugular vein and puncturing the brain. Immediately after they were killed, X-ray shadowgraphs were made of the legs in order that the calcification of the bones might be studied by this means. The leg bones were then removed and dissected free of flesh in preparation for ashing. The epiphyseal cartilages were removed from the bones of one-half of the birds but not from the bones of the other half. Thus, one-half of the samples were made up of the diaphysis alone, and the other half included the diaphysis and the epiphyses. The long bones (femur, tibia with fibula, and metatarsus) of the left leg were combined in one sample, whereas the femur, tibia without fibula, and metatarsus of the right leg were studied separately.

During the first 6 weeks, the following numbers of birds were used in preparing each sample at the ages indicated.

Number of birds represented in each sample:	Age
15	1 day.
12	1 week.
8	2 weeks.
7	3 weeks.
6	4 weeks.
6	5 weeks.
5	6 weeks.

After the age of 6 weeks, one bird was sufficient for the weekly sample.

Bone samples of chicks of each sex were obtained as soon as the males could be readily distinguished from the females. As each bone was freed of flesh and its periosteum, it was placed in 95-percent ethyl alcohol and kept there until the determination of its ash content was begun. The following method of obtaining the ash content of the bones was used: The bones were removed from the alcohol, cut into small pieces with bone snips if they were inconveniently large, wrapped in filter paper, and extracted for 6 hours with 95-percent ethyl alcohol in a Soxhlet extractor. They were next allowed to dry in the open air and then extracted with diethyl ether for 6 hours. After being dried again in the air, the bones were placed in weighed crucibles, dried in a vacuum oven for 4 hours at a temperature of 60° C., and weighed. The weighed, oven-dried samples were then ashed to a constant weight, at 600°, to obtain the weight of ash in the moisture- and fat-free bones.

Previous, but unpublished, work at the United States Animal Husbandry Experiment Farm has shown that this method is efficient from the standpoint of accuracy and rapidity of determination. The extraction should be continued only long enough to insure removal of the soluble material. For this purpose, the writers have found 6 hours with each solvent to be sufficient, for the percentage of ash after such treatment was fully as high as that reported by St. John and his coworkers (*9*) when much longer periods were employed.

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[2] Reference is made by number (italic) to Literature Cited, p. 1008

The amount of calcium and phosphorus in the ash of the tibiae was then obtained.[3] For this purpose, the ash was dissolved in dilute hydrochloric acid and the resulting solution was made up to a definite volume. Aliquot parts of this solution were used for the determination of calcium according to Kramer and Howland's (*8*) method for bone, and for the determination of phosphorus by the gravimetric method of the Association of Official Agricultural Chemists (*1*) for fertilizers.

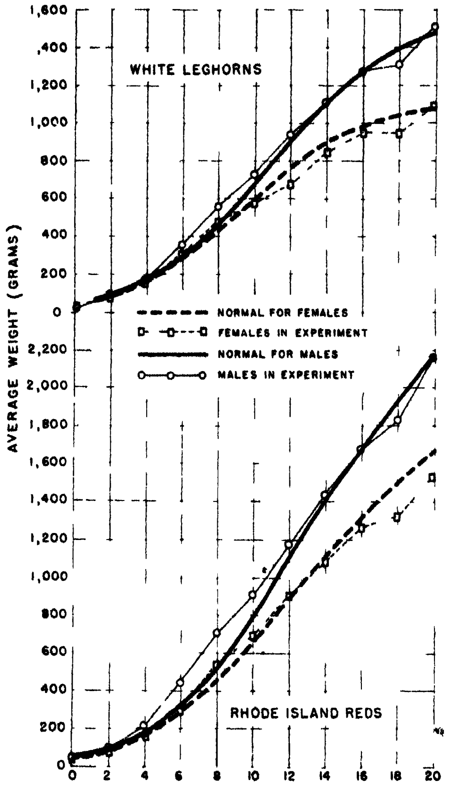

FIGURE 1.—Average weights, at 2-week intervals, of males and females of the two breeds compared with normal weights reported for these breeds by Hendricks, Lee, and Titus (*5*) and by Titus and Jull (*10*).

EXPERIMENTAL RESULTS

GROWTH OF THE BIRDS

The average growth (as measured by weight in grams) of the birds which were banded is shown in figure 1. There were 35 males and 44 females of the White Leghorn breed and 37 males and 45 females of the Rhode Island Red breed The curves show that the growth was satisfactory as compared with the growth previously obtained at the Beltsville experiment farm with these breeds (*5*, *10*).

CALCIUM AND INORGANIC PHOSPHORUS CONTENTS OF BLOOD SERUM

The calcium and inorganic phosphorus contents of the blood serum of the birds at intervals of 1 week are shown in figure 2. All the values obtained were well within the normal limits, as shown by unpublished results; in fact, there was less variation than is frequently observed under experimental conditions. The variation was so small throughout most of the first 20 weeks as to have no apparent significance. Shortly after the reduction of the relative quantities of the protein concentrates in the diet of the birds at the age of 14 weeks, there was a decrease for about 4 weeks in the inorganic phosphorus content of the blood serum, whereas the calcium content showed no change. This decrease in phosphorus content was then followed by an increase for 2 or 3 weeks and then by another decrease. There was, apparently, no significant difference in the calcium and inorganic phosphorus contents of the blood serum of the males and females from the sixth or seventh week, when sex was first differentiated, to the twentieth week.

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[3] The determinations of the calcium and phosphorus in the ash of the tibiae were carried out by R. E. Davis and G. H. Kennedy, of the Animal Husbandry Division.

Determinations of the calcium and inorganic phosphorus in the blood serum of the birds were continued from the ages of 20 to 25 weeks, with the special object of observing the changes in the calcium of the blood serum of the pullets as they began to lay, as compared with those changes in the blood serum of the males of the same ages. Figure 2 shows a very marked increase in the case of the White Leghorn pullets after the twentieth week and a similar increase in the case of the Rhode Island Red pullets after the twenty-third week. This increase was undoubtedly due to the increased demand for calcium to be used in egg production, as the pullets began to lay at about this time. Halnan (*4*) has shown that there is an increased retention of calcium from the feed during the week preceding the beginning of egg production, and Hughes and his coworkers (*7*) have found that there is an increased content of calcium in the blood serum of pullets at the time that laying begins.

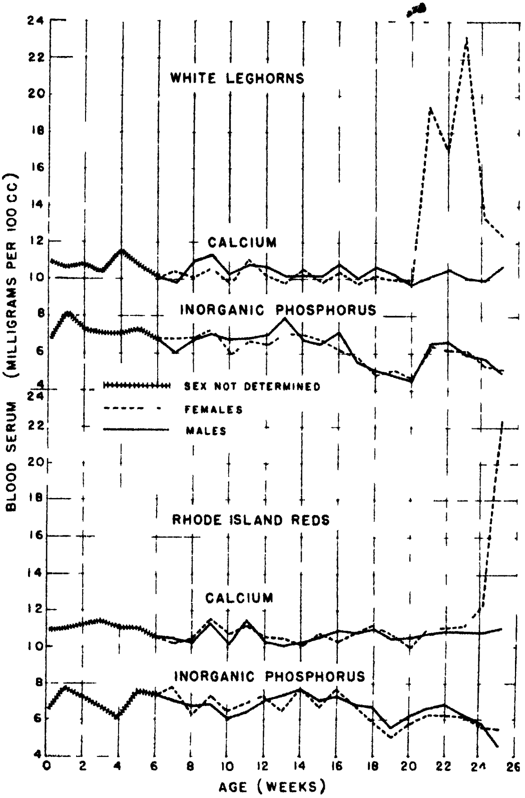

FIGURE 2.—Calcium and inorganic phosphorus contents of the blood serum of White Leghorns and Rhode Island Reds at weekly intervals.

CALCIFICATION OF EPIPHYSES

Through an examination by the X-ray shadowgraphs, certain stages in the calcification of the epiphyses were noted. During the first week of the life of the chick, centers of ossification appeared in the cartilages at the distal end of the tibia and the proximal end of the metatarsus. There was no difference between the two breeds in this respect. Centers of ossification appeared in the cartilage at the proximal end of the tibia by the end of the sixth week. In this respect also no difference was noted between the two breeds.

After the first appearance of the centers of ossification the areas undergoing calcification gradually increased in size until they made up most of the epiphyses and the epiphyses became attached to the diaphysis. The union of a diaphysis with an epiphysis occurred first at the proximal end of the metatarsus, then at the distal end of the tibia, and later at the proximal end of the tibia. There seemed to be a tendency for the epiphyses of the females to calcify at an earlier age than those of the males, and for those of the White Leghorn birds to calcify at an earlier age than those of the Rhode Island Reds (table 1).

TABLE 1.—*Effect of breed and sex on the age at which the calcification of the epiphyses were apparently complete, as shown by the X-ray shadowgraphs*

Breed and sex of birds	Age of bird when calcification occurred at—		
	Proximal end of metatarsus	Distal end of tibia	Proximal end of tibia
	Weeks	*Weeks*	*Weeks*
White Leghorn:			
Female	12	13	19
Male	14	15	20
Rhode Island Red			
Female	13	17	20
Male	14	19	(a)

[a] Not complete at twentieth week.

ASH CONTENT OF BONES

The percentage of ash in the moisture- and fat-free bones each week of the experiment is presented in figures 3 and 4, and the percentages

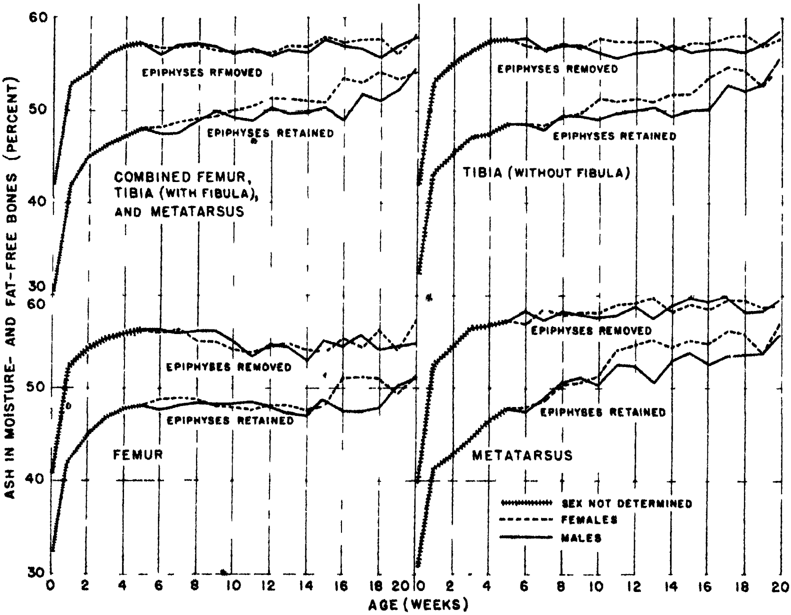

FIGURE 3.—Ash content of the bone samples obtained from the White Leghorns at weekly intervals

at 4, 5, 6, 7, 8, and 20 weeks are given in table 2. The curves are comparatively smooth during the first few weeks, when the determinations of ash were made on samples composed of bones of from 5 to 15 individuals. Later, when the samples represented single birds, these curves are not so smooth.

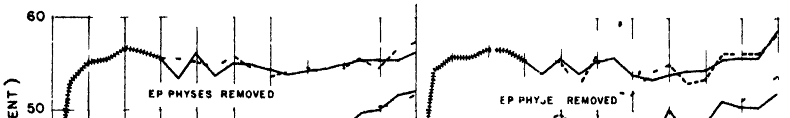

Figure 4.—Ash content of the bone samples obtained from the Rhode Island Reds at weekly intervals

Table 2.—*Percentage of ash in the moisture- and fat-free bones of the White Leghorns and the Rhode Island Reds at the ages indicated*

WHITE LEGHORNS

Age in weeks	Sex	Bones with cartilage retained: Femur	Tibia	Metatarsus	Combined bone	Bones with cartilage removed: Femur	Tibia	Metatarsus	Combined bones
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4	Not separated	47.8	47.5	46.4	47.3	55.9	57.4	56.7	56.9
5	do	48.2	48.4	[illegible]	48.1	56.1	57.6	57.2	57.1
6	Male	47.8	48.7	47.0	47.6	56	57.8	58.	56.0
	Female	48.8	48.6	47.6	48.1	56.0	57.1	56.8	56.9
7	Male	48.2	48.1	49.0	47.6	56.0	56.4	57	57.1
	Female	48.8	48.1	48.6	48.8	56.2	56.7	58.1	56.8
8	Male	48.5	49.5	50.8	49.6	56.0	57.0	58.3	57.2
	Female	48.7	49.1	50.5	49.0	55.1	57.3	57.9	56.9
20	Male	50.9	55.8	55.9	51.4	51.8	59.6	59.4	57.9
	Female	51.2	54.6	56.8	54.3	57.3	57.7	58.8	58.2

RHODE ISLAND REDS

Age in weeks	Sex	Bones with cartilage retained: Femur	Tibia	Metatarsus	Combined bone	Bones with cartilage removed: Femur	Tibia	Metatarsus	Combined bones
4	Not separated	46.7	47.6	46.5	46.5	55.0	56.6	57.0	56.7
5	do	46.3	46.8	46.5	46.6	55.3	56.4	56.9	56.5
6	do	46.4	47.0	46.1	47.3	55.3	55.4	56.3	55.6
7	Male	45.0	46.1	45.5	45.9	52.2	54.0	54.6	53.5
	Female	47.1	49.0	48.3	48.0	54.6	55.4	56.8	55.5
8	Male	45.0	47.0	46.9	46.6	53.7	55.8	56.6	56.1
	Female	47.4	49.8	49.7	49.1	54.4	55.4	55.8	55.1
20	Male	50.0	51.8	54.4	52.2	52.6	58.5	58.3	56.0
	Female	49.0	53.6	56.6	51.8	55.3	58.5	59.8	57.4

In general, the ash content of the moisture- and fat-free bones of the chicks at the age of 1 day was approximately 40 percent when the epiphyseal cartilages were removed and 30 percent when they were retained. There was a marked increase in the ash content during the first week, and this increase continued, but at a progressively slower rate, during the next few weeks. Thereafter, there was relatively little change.

A comparison of the curves representing the ash content of the bones, with and without the epiphyseal cartilages, shows that the diaphysis contains an appreciably higher percentage of ash than does the diaphysis with the epiphyses. This difference is greatest during the first few weeks, after which the curves representing the percentage of ash in the bones prepared in the two different ways tend to approach each other gradually and are fairly close together at the end of 20 weeks.

The ash content of the bones obtained from the White Leghorns was higher than that of the corresponding bones of the Rhode Island Reds. This difference continued throughout most of the period of 20 weeks covered in the present study. After approximately the nineteenth week, however, there was a tendency for the ash content of the bones of the Rhode Island Reds to become very nearly equal to that of the bones of the White Leghorns. Thus it appears that calcification of the leg bones proceeds at a higher rate and is completed at an earlier age in the White Leghorns than in the Rhode Island Reds. Such a difference is to be expected, since the former matures somewhat more rapidly than the latter.

Another point brought out by these curves is that throughout most of the period when the males and females were studied separately the average ash content of the leg bones of the females from which the cartilage had not been removed was higher than that of the leg bones of the males. However, at the twentieth week there was, in general, very little difference. In contrast to this, the ash content of the bones from which the cartilage had been removed was practically the same for the two sexes.

A comparison of the three long bones of the leg shows that the metatarsus had the most ash, the femur the least, and the tibia an intermediate amount. During the preparation of the samples a corresponding difference was noted in the physical character of these three bones. The metatarsus was yellowish white in color, had a yellow marrow, greater strength, and less brittleness than either of the other two bones. There was less difference between the femur and the tibia. Both of them were grayish white, had a red marrow, and tended to shatter readily when cut with the bone snips, in contrast to the metatarsus which permitted the bone snips to cut through cleanly. The femur, however, seemed to be somewhat more brittle than the tibia. Thus, it appears that the ash content of the bones bears some relation to their physical character.

CALCIUM AND PHOSPHORUS CONTENTS OF THE ASH OF THE TIBIA

There was relatively little change in the calcium and phosphorus contents of the ash of the tibiae throughout the 20 weeks and, for this reason, only the averages of the results obtained are given in table 3. After the first week the values ranged between approximately 34.5 and 37 percent of calcium and 17.5 and 18.5 percent of phosphorus. There was apparently no difference in this respect between the bones of cockerels and pullets or between birds of different ages.

TABLE 3.—*Effect of age and sex on average percentage of calcium and phosphorus in the ash of the tibiae of White Leghorns and Rhode Island Reds*

Age of birds	Sex of birds	Bones with cartilage retained			Bones with cartilage removed		
		Ca	P	Ca/P ratio	Ca	P	Ca/P ratio
WHITE LEGHORNS							
		Percent	*Percent*		*Percent*	*Percent*	
1 day	Not separated	31.3	17.7	1.77:1	35.0	20.4	1.72:1
1-5 weeks	do	35.0	18.1	1.94:1	34.2	17.8	1.94:1
6-20 weeks	Male	35.7	17.9	1.98:1	36.7	18.2	2.02:1
	Female	35.9	17.6	2.05:1	36.2	18.1	2.00:1
RHODE ISLAND REDS							
1 day	Not separated	33.4	18.9	1.77:1	35.0	19.8	1.77:1
1-6 weeks	do	34.5	17.9	1.93:1	34.8	17.9	1.96:1
7-20 weeks	Male	35.3	17.8	1.97:1	36.1	18.1	1.99:1
	Female	36.7	18.4	2.00:1	36.7	18.4	2.00:1

The ratio of calcium to phosphorus in the ash of the tibiae was found to be very close to 2.0:1 in most of the samples; therefore the averages of the values, given in table 3 for the different breeds and sexes, in most instances are also very close to 2.0:1. However, one exception to the foregoing statement is that of the calcium-phosphorus ratio of the ash of the tibiae in birds aged 1 day. The average ratio was 1.77:1 in three cases and 1.72:1 in the fourth.

DISCUSSION

The data presented in this paper on the ash content of the moisture- and fat-free leg bones of chickens indicate that there was little tendency for the percentage of ash in those bones from which the cartilage had been removed to change after the fourth or fifth week. There appears, however, to have been a slight tendency for the ash content of the metatarsus to increase with the age of the chick and for that of the femur to decrease between the fifth and fifteenth week. Nevertheless, in both cases the maximum deviation from the average ash content observed between the seventh and twentieth week was less than 4 percent of the average.

Although the data show that the ash content of the bones from which the epiphyseal cartilages had been removed tended to remain rather constant from the fourth to the twentieth week, they also show that there was a gradual increase, during this period, in the ash content of the bones (other than the femur) from which the cartilages had not been removed. It appears, therefore, that this change was due to a progressive calcification of the epiphyseal cartilages. In any case, the ash content of the bones from which the cartilages had not been removed tended to become more nearly equal to that of the bones from which the cartilages had been removed as the birds approached maturity and the calcification of the epiphyses became more nearly complete.

The tendency for the ash content of the diaphysis to remain rather constant under normal conditions does not necessarily mean that the ash content may not change under certain conditions, for unpublished experiments by the writers have shown that it may change either as the result of the occurrence of rickets or because of a change in the rate of growth. If the rate of growth is slow, the cartilage-free bones may have an abnormally high ash content. On the other hand, very rapid growth, especially after a period of slow growth, tends to bring about a decrease in the ash content of both the diaphysis and the epiphyseal cartilage. It is quite probable that much of the variation among individual chickens of the ash content of their leg bones is due largely to differences in their rate of growth.

It is necessary, therefore, to consider the rate of growth of the chickens in any experimental or regulatory work with feeds in order that errors in the interpretation of results may not occur. Thus it is possible that a diet containing a cod-liver oil low in vitamin A might contain an inadequate supply of this vitamin. The chickens receiving such a diet would tend to grow more slowly than others receiving an adequate supply, and probably the percentage of ash in their bones would be higher than if their growth had been normal. Failure to take the growth rate into account would lead to the conclusion that the antirachitic value of the oil under consideration was higher than was actually the case.

The results of the present study indicate that, in most cases, when the birds are between the ages of 7 and 20 weeks, the ash content of the females' bones with the cartilage retained is greater than that of the males' bones. The X-ray shadowgraphs furnish evidence that this difference is due to an earlier calcification of the epiphyses of the bones of the females rather than to a greater storage of minerals in preparation for egg production, as was suggested by Holmes, Pigott, and Moore (*6*). Furthermore, the bones of the males, either with or without their cartilages, contain approximately the same proportion of ash at the age of 20 weeks as do those of the females. Moreover the greater weight of the bones of the males, as is evident from their greater body weight, indicates a larger total quantity of ash stored in their skeletons.

Since the above-mentioned difference was found to be due to an earlier calcification of the cartilages in the case of the females, it can be avoided in experimental studies by removing the epiphyseal cartilages from all bones to be ashed. Furthermore, if the epiphyseal cartilages are removed in preparing the samples, the writers' results indicate that it is unnecessary to consider the sex of the individual or its age, between the fifth or sixth and the twentieth week. The removal of the cartilages in the preparation of the bone for ashing makes the dissection of the bone easier and more accurate, for the cartilages can be removed very readily with the flesh and periosteum. The accuracy of the dissection is an important point, for the writers have found, in unpublished work, that there is more danger of error resulting from faulty dissection than from any other step in the analytical technic.

The objection may be raised that the diaphysis alone would not indicate the extent of the occurrence of rickets so well as would the diaphysis together with the epiphyseal cartilages. St. John, Kempf, and Bond (*9*) found that between normal and rachitic chicks there was a greater difference in the ash content of the epiphyseal cartilages than in that of the diaphyses. Although this is true, it is also true that after the first few weeks the epiphyses make up only a small portion of the combined weight of the bone and cartilages and would, therefore, have less influence on the ash content of the sample than would the diaphysis. The writers have found, in unpublished data, that the ash content of the diaphyses of bones obtained from rachitic chicks is almost as low as the ash content of the diaphysis and epiphyses combined. They conclude, therefore, that it is entirely satisfactory to use the percentage of ash in the diaphyses of the leg bones as a measure of the extent of the development of rickets in the chicken.

The data obtained by the writers seem to indicate that the tibia would ordinarily be most satisfactory for determining the ash content of the leg bones of chickens reared under normal conditions. The ash content of the tibia agrees closely with that of the combined long bones of the leg, and the use of the tibia alone has the advantage of simplifying the analytical procedure. This bone has also been generally used in the work reported in the literature.

SUMMARY AND CONCLUSIONS

Two groups of chickens, one consisting of 350 Single-Comb White Leghorns and the other of 350 Rhode Island Reds, were reared on a grass range under normal conditions. The ash content of the moisture- and fat-free leg bones of representative individuals from each group was determined each week during the first 20 weeks of their lives. Stages of calcification of the epiphyses in the leg bones of these birds were noted by means of X-ray shadowgraphs. The contents of the calcium and inorganic phosphorus of the blood serum and of the ash of the tibiae also were obtained.

There was no significant change in the calcium content of the blood serum of the birds with increasing age, except for a marked increase in the case of the pullets just before they commenced to lay. After the fourteenth week there were marked changes in the inorganic phosphorus content of the blood serum, which were probably due in part to increasing age and in part to a change in the diet which was made at that time.

The X-ray shadowgraphs indicated an earlier calcification of the epiphyses of the bones of the females than of the males and also an earlier calcification of the epiphyses of the bones of the White Leghorns than of the Rhode Island Reds.

There were no significant differences in the calcium and phosphorus contents of the ash of the tibiae, nor was the difference significant between the sexes. The ratio of calcium to phosphorus in the ash of the tibiae was noticeably low on the day after hatching, after which it increased to approximately 2.0:1 in all cases.

The ash content of the diaphysis of the long bones of the legs of chickens of both sexes tended to remain constant between the fifth and twentieth weeks. In general, the ash content of the diaphysis with its cartilages was higher in the case of the females than in the case of the males between the sixth or seventh and the eighteenth week, depending on the breed.

It is concluded that the tibia is the most satisfactory bone to use in studying the effect of various diets and sources of vitamin D on the ash content of the bones of chickens. It is recommended that the epiphyseal cartilages, together with the periosteum, be removed in the preparation of the bone for ashing.

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THE LETHAL EFFECT OF LOW TEMPERATURES ON THE VARIOUS STAGES OF THE CONFUSED FLOUR BEETLE[1]

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INTRODUCTION

Entomologists know that stored-product insects are not very resistant to low temperatures. Refrigeration is employed commercially to prevent insect damage. Ordinarily, insect activity is suspended in infested articles, or the infestation by insects is prevented in "sterilized" goods. However, low temperature is rarely used as the sterilizing agent in place of heat or a fumigant.

As compared with the attention paid to the effect of high temperature, but little investigation has been made of the lethal effect of low temperatures. It must be recognized that the cold resistance of different species of insects may vary widely, whereas the heat resistance varies only slightly. In order to obtain the most economical combination of the temperature and the length of exposure, actual data are necessary regarding the time required to kill a certain insect species at any given low temperatures.

Because the confused flour beetle (*Tribolium confusum* Duval) is an insect with which prepared food products are likely to become infested, it was selected for the study reported in this paper. The data presented are of particular interest also for their physiological significance.

In applying time-temperature data to the effect of low temperatures on insects, it should be understood that allowance must be made for the time necessary to cool the insect-containing medium, as, for example, wheat flour. The measurement of the cooling (heat transfer) time of standard volumes of various commercial products is a separate study which will not be considered here.

HISTORICAL REVIEW

Most of the data available in the literature regarding the lethal effects of low temperatures on stored-product insects are merely isolated observations, often presented without the time of exposure being reported. The first accurate measurement of the effect of refrigeration on an insect was made by Back and Pemberton (*1*, *2*),[2] who found it possible to kill the early stages of the Mediterranean fruit fly by cold storage.

Although some data published relative to this subject are available for other stored-product insects, only one reference to the effect of low temperatures on *Tribolium confusum* is known. Payne (*8*) says that normally it may recover from temperatures as low as - 14° C., but she makes no mention of the duration of the exposure.

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[1] Received for publication Jan 4, 1934, issued July 1934 Paper no 1235 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station

[2] Reference is made by number (italic) to Literature Cited, p 1016

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EXPERIMENTAL METHOD

A Carrier air-conditioned cabinet was employed in the experiments involving temperatures from 0° to −18° C. A variation of less than 1° was obtained for the period of each experiment. For the experiments in which higher temperatures were used another type of cabinet was utilized in which the temperature varied only slightly over a period of several weeks.

Tribolium confusum were cultured in fine whole-wheat flour, about 500 grams for each lot. The flour was sifted through a standard no. 6 silk bolting cloth (74 meshes to the linear inch). About 3,000 adult beetles were placed in each lot. The cultures were covered with muslin and kept at a constant temperature of 27° C. Eggs were seldom allowed to accumulate more than 24 hours.

All stages except the adults were exposed in small glass vials, 50 individuals in each, with a few exceptions when 100 were so exposed. The eggs were counted under a binocular microscope, only those which were normally plump being used. The unstoppered vials with their contents were placed in the temperature cabinets. After exposure each vial was lightly stoppered with cotton or with cork stoppers having screen-covered holes. Adults were exposed in silk bolting-cloth cylinders. This precaution was necessary because preliminary tests had shown that the excited adults give off an odoriferous vapor. When confined with this gas they soon die, 95 percent being killed in 4 or 5 hours.

During the earlier experiments exposed eggs without flour were placed in a cabinet and kept at a temperature of 27° C. During the hatching, results were noted daily, the young larvae being removed from the vials and discarded. Later tests showed that the results were the same if eggs were allowed to incubate in about 2 grams of flour and the larvae counted 3 or 4 days after hatching began. The tedious work of noting results was thus greatly reduced. All other stages of the insect were treated in this manner. Check experiments of 50 individuals each were always included in each series. They were kept at 27° and their condition noted only after the observations on the exposed insects had been completed.

All experiments at 7° C. were carried out in glass desiccators at three different relative humidities, viz. 6.2 (7-mm saturation deficiency), 50, and 73.7 percent. With the exception of five experiments which were made without moisture control, all experiments at 12° were carried out likewise in desiccators at relative humidities of 32.8 (7-mm saturation deficiency), 50, and 73.7 percent. At both temperatures no differences in the lethal effects at different humidities were observed, neither did there appear to be any difference in the results obtained from the experiments conducted outside the desiccators. Accordingly, all the experiments at each of these temperatures were grouped without regard to moisture conditions.

The percentage of mortality in each case was calculated by Abbott's formula, $\frac{x-y}{x}(100)$, where x is the percentage survival in the check and y that in the exposed lot. In figure 1 are shown curves for eggs of three age groups at 12° C. Fifty and 100 percent mortalities were estimated in all cases from curves of this type. Each point in the curve shown is an average of the results of from 1 to 14 experiments, each involving 50 individual insects. Although a few points are based on a single experiment, no curve is drawn through less than eight points.

INFLUENCE OF AGE ON COLD RESISTANCE OF EGGS

Davis (7), studying the effects of high and low temperatures upon the mosquito *Aedes aegypti* Linn., found that at −5.5° C., freshly laid eggs were slightly more resistant than mature eggs. The writers have found the same thing to be true for eggs of *Tribolium confusum* that have been exposed to chloropicrin.

In order to study the effect of age on the resistance of eggs of *Tribolium confusum* to low temperature, eggs were placed in six age groups, namely, 1 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, and 120 to 132 hours old. At 27° C., the temperature at which the sifted eggs were stored, those 132 hours old were nearly ready to hatch. Eggs in the different age groups were simultaneously exposed to low temperatures and otherwise treated as nearly alike as possible. In counting the survivors of these tests, only those larvae which previous experience showed to be able to complete their development were counted. Certain larvae died after emerging partly from the egg. Others hatched in such a weakened condition that they also soon died. All such larvae were considered as having been killed in the egg stage. The eggs set aside as checks showed from 78.38 to 90 percent survival as calculated from the number of young larvae hatched from them.

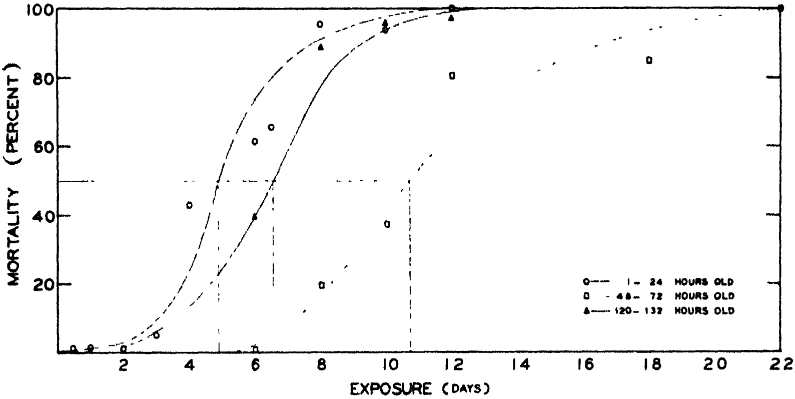

FIGURE 1.—Mortality of eggs of *Tribolium confusum* of different ages when exposed to a temperature of 12° C.

Figure 1 shows the average results at each age-temperature combination. In table 1 is given a summary of the time required at the various temperatures to obtain 50 and 100 percent mortality. The latter figures do not possess, for comparative purposes, the precision of the previous ones, but they are more useful in estimating time-temperature relations for insect control. Although eggs were separated into 6 age groups most of the experiments were confined to 3 ages, namely, 1 to 24, 48 to 72, and 120 to 132 hours old. A few results with eggs at other ages are given for the sake of showing the influence of the entire range of age on the lethal effects of temperature at −12° C.

TABLE 1.—*Effect of low temperatures on eggs of Tribolium confusum of different ages, 50 and 100 percent mortality being the basis of comparison*

50 PERCENT MORTALITY

Temperature (° C.)	Number of hours of exposure required to produce mortality in eggs of—					
	Age 1 to 24 hours	Age 24 to 48 hours	Age 48 to 72 hours	Age 72 to 96 hours	Age 96 to 120 hours	Age 120 to 132 hours
12	118		258			157
7	43		214			110
0	4.75	4.5		11.25		
−4	1.5	1.5	3.5	3.75	2.75	
−6	.75		3.25			4
−12	.75	1.5	3.5	6	2.75	1.5
−12 [a]	.75		.75			1.25
−18	.25		.25			.25

100 PERCENT MORTALITY

Temperature (° C.)	Age 1 to 24 hours	Age 24 to 48 hours	Age 48 to 72 hours	Age 72 to 96 hours	Age 96 to 120 hours	Age 120 to 132 hours
12	288		528			312
7	216		432			288
0	13	11.5		16	13.5	
−4	9.25	8.25	7.5	9.5	8.25	
−6	6.5		8			13
−12	9.5	9.5	14	12	9.5	6
−18	1		1			1

[a] The second series of experiments at −12° C. included fewer eggs for the determinations at 1 to 24 hours and at 48 to 72 hours than in the first series at that temperature.

The results show that in general the resistance of the eggs is probably greatest in the fourth age group which includes eggs 72 to 96 hours old. According to available embryological data for *Tribolium confusum* at this age, the embryos have just undergone dorsal closure and the young larvae are becoming rather well formed. It is known for insect embryos in general that their respiration is at a lower level here than at other stages of their development.

INFLUENCE OF AGE ON COLD RESISTANCE OF LARVAE

For the purposes of the experiments with larvae of *Tribolium confusum* three age groups were selected. The first group consisted of unfed larvae 1 to 12 hours old, averaging 6 hours. The second and third groups of about half-grown and of full-grown larvae, respectively, were raised on whole-wheat flour, from eggs the age of which was known for each lot. Because larvae of the same age varied in size considerably under the culture conditions, a few third- and sixth-instar larvae were selected by the aid of the measurements given by Chapman (*5*) and by Brindley (*3*). Then by comparison it was possible to select with a fair degree of accuracy the larvae of these instars from the cultures in which they predominated. Only active larvae able to resist jarring from a sheet of paper were used, thus eliminating those in the process of molting.

The manner in which the larvae were handled was practically the same as that for the eggs. Fifty individuals were used in each experiment. Observations were made regularly 1 week after treatment. The groups of larvae set aside as checks showed 91.2 to 100 percent survival.

In table 2 is summarized the time in hours required to obtain 50 and 100 percent mortality for the three age groups of larvae at different low temperatures. These data indicate that the full-grown larvae are most resistant, although the difference is pronounced only at 7° C.

Larvae killed outright by freezing invariably turn black soon afterward. Some others turn black in irregular areas. Many larvae which otherwise appeared normal were unable to void feces properly. In some cases long, sticklike strands protruded from the anus a distance equivalent to the length of the entire body. In full-grown larvae certain time-temperature combinations produced rare winged freaks, examples of metathetely.[3]

TABLE 2.—*Effect of low temperatures on larvae of Tribolium confusum at different stages of growth, 50 and 100 percent mortality being the basis of comparison*

50 PERCENT MORTALITY

Temperature (° C.)	Number of hours of exposure required to produce mortality in larvae of—		
	Age 1 to 12 hours	Third instar	Sixth instar
7	134.4	149	197
−6	10	9	11.75
−12	42	.35	48
−18	.18	.17	17

100 PERCENT MORTALITY

Temperature (° C.)	Age 1 to 12 hours	Third instar	Sixth instar
7	288	250	528
−6	16	16	16
−12	1.75	5	1
−18	1	5	.5

EFFECT OF LOW TEMPERATURES ON PUPAE

Pupae of only one age were selected. In general, the lighter-colored ones are youngest, and these were chosen for use in the tests. Each lot of treated pupae was placed in about 2 grams of flour at 27° C. Mortality was based on the percentage emergence of the adults. The groups of pupae set aside as checks showed a high proportion of emergence, 96.32 to 100 percent.

In table 3 are summarized the times in hours required to obtain 50 and 100 percent mortality of pupae at different low temperatures. Results at 12° C. are not tabulated because exposures over a long time showed very little mortality. The keeping of 100 pupae at 12° for 22 days resulted in only 4 dead specimens. No adults emerged during this long exposure.

TABLE 3.—*Effect of low temperatures on pupae of Tribolium confusum*

Temperature (° C.)	Hours of exposure required to produce—	
	50 percent mortality	100 percent mortality
7	258	432
−6	.67	10
−12	.63	1.5
−18	.13	.5

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[3] Chapman (*6, p. 294*) refers to Strickland's study of parasitized *Simulium* larvae in which Strickland found that the action of the parasite prevented the wing histoblasts from developing normally, the wing pads being greatly retarded and reduced. To this phenomenon, Strickland gave the term "metathetely" (to run behind completion).

EFFECT OF LOW TEMPERATURES ON ADULTS

Adults ranging in age from 1 to 5 months were used, no attempt being made to study the effect of age on their resistance to low temperatures. As the adults live a year or more, those used were young and vigorous. Treated adults were kept under observation for a week or so without flour. Check groups of adults under these conditions showed 94.66 to 96 percent survival.

In table 4 are summarized the times in hours required to obtain 50 and 100 percent mortality of adults at different low temperatures. Exposures of adults at 12° C., as in the case of pupae at this temperature, are not tabulated. Some activity and little mortality of adults occurs at this temperature, groups being kept at different humidities for from 22 to 35 days without showing over 6 percent mortality.

TABLE 4.—*Effect of low temperatures on adults of Tribolium confusum*

Tempera- (° C)	Hours of exposure required to produce—	
	50 percent mortality	100 percent mortality
7	336	528
− 6	8.4	24
−12	.23	2
−18	15	.5

As mentioned before, it was necessary to place the adults for treatment in bolting-cloth cylinders to prevent accumulation of the toxic vapors given off by the excited beetles. Adults injured by low temperature also give off this vapor which, if the beetles are placed in flour, imparts a definitely pink color to that flour. Several series of experiments indicated that the intensity of this color varied directly with the length of exposure to low temperature. Chapman (*6*) noted this color in flour and other materials, stating that the vapor is given off by beetles irritated mechanically.

Adults not injured sufficiently to die as a result of their cold treatment were counted as survivors. It was found that survivors could usually be easily determined after a week was allowed for recovery. In two instances adults exposed to −12° C. for 45 minutes were still alive after 45 days, but they were never able to crawl about. Inability to void feces properly was common among injured adults as in the case of the larvae. Whenever the recovery of an individual was in doubt, it was counted as a survivor. Some living adults, although more or less paralyzed after they had been exposed to −12° for 45 minutes, produced fertile eggs. Survivors of 6 hours exposure to −6° produced many fertile eggs. Four hundred adults were kept in 300 grams of flour at 12° for 29 days, but no eggs were laid at that temperature. Several days after the removal of these adults from the low temperature 400 to 500 eggs were recovered, indicating that egg laying was normal after such a period of inactivity.

RELATIVE RESISTANCE OF DIFFERENT STAGES

Table 5 shows the time-temperature dosages required to kill both 50 and 100 percent of the different stages of the confused flour beetle. In the case of the eggs and the larvae, several ages of which were exposed to low temperatures, the results for the most resistant ages are given.

TABLE 5.—*Effect of low temperatures on the various stages of Tribolium confusum 50 and 100 percent mortality being the basis of comparison*

50 PERCENT MORTALITY

Stage	Number of hours of exposure required to produce mortality at temperature of				
	7° C	0° C	−6° C	−12° C.	−18° C.
Egg	214	11.25	4	3.5	0.25
Larva	197		11.75	48	18
Pupa	258		67	63	.13
Adult	336		8.4	.23	15

100 PERCENT MORTALITY

Stage	7° C	0° C	−6° C	−12° C.	−18° C.
Egg	432	16	14	14	1
Larva	528		16	1.75	1
Pupa	432		10	1.5	5
Adult	528		24	2	5

It is apparent from the figures for 50 percent mortality that the adults are more resistant than the other stages at the moderately low temperature of 7° C. At −6°, however, the larvae are about as hard to kill as the adults and at −12° the eggs are by far the most resistant. The pupae succumb more readily than the other stages.

The figures for 100 percent mortality provide answers to the practical questions of how low a temperature and how long an exposure is necessary to kill all stages of the confused flour beetle. It is possible to kill every stage of the insect at 7° C. (44.6° F.) if held at that temperature for nearly 25 days (600 hours). Only 24 hours' exposure is necessary if the temperature can be lowered to a little below −6° C. (21.2° F.).

DISCUSSION

In no previous instance apparently have the effects of low temperatures on insects been compared on the basis of 50 percent mortality. Carter (*4*) compared the resistance of various stages of the bean weevil (*Mylabris obtectus* Say) on the basis of 100-percent kill. All stages were killed only after several hours exposure to temperatures of −12° to −15° C., whereas *Tribolium confusum* in every stage but the egg succumbs in 2 hours or less at −12°. Robinson (*9*) determined the resistance of the rice and the granary weevils (*Sitophilus oryzae* L. and *S. granarius* L.) to various low temperatures at intervals from their respective thresholds of activity to as low as −17.7°. From a comparison of his data with those given here for *T. confusum* it appears that the latter species is nearly as susceptible to low temperatures as the rice weevil. Given the same low temperature in their environments, it is much easier to kill the confused flour beetle with cold than it is the granary weevil. Robinson reports that *S. granarius* requires 100 hours at −6.6° for a complete kill, whereas it has been shown here that all stages of *T. confusum* are killed at −6° in 24 hours.

SUMMARY

A study was made of the lethal effect of low temperatures on four stages of the confused flour beetle (*Tribolium confusum* Duval)—egg, larval, pupal, and adult. Data were obtained showing the number of hours of exposure required to produce 50- and 100-percent mortality, the temperatures ranging from 12° to −18° C.

The eggs were placed in six groups according to age, 1 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, and 120 to 132 hours. Resistance to cold was found to be greatest in the fourth age group, 72 to 96 hours.

The larvae were placed in three age groups—unfed larvae 1 to 12 hours old, half-grown, and full-grown larvae. The full-grown larvae were found to be the most resistant, although the difference was pronounced only at 7° C.

The mortality of the pupae was based on the percentage of emergence of the adults.

The adults ranged in age from 1 to 5 months, and those not injured sufficiently to die as a result of exposure to low temperatures were counted as survivors.

The figures for 50-percent mortality show that the adults were more resistant than the other stages at 7° C.; at −6° the larvae were about as resistant as the adults; and at −12° the eggs were the most resistant.

The figures for 100-percent mortality show that all stages of the insect can be killed at 7° C. if exposed at that temperature for 25 days; but if the temperature is lowered to −6°, only 24 hours' exposure is necessary.

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INCREASE OF KERNEL WEIGHT IN COMMON WHEAT DUE TO BLACK-POINT DISEASE[1]

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INTRODUCTION

This paper deals mainly with the relative weights of kernels of wheat (*Triticum vulgare* Vill.) showing the presence of "black point" and those free from this infection. The main study was made upon kernels from individual wheat plants from the F_5 population resulting from the cross Reward × ((Kota × Marquis) × Hope). The wheat, grown at Fargo in 1933, was planted on May 5, which was about 2 weeks later than the earliest date available for planting in the nursery where it was grown.

A sample of the infected kernels was submitted to H. B. Humphrey, of the Bureau of Plant Industry, United States Department of Agriculture. Dr. Humphrey reported that about one half of the kernels were infected with a strain of *Alternaria*, while the remainder were clearly and positively infected with *Helminthosporium sativum* Pam., King, and Bak. Black point was much in evidence in a number of common wheats in 1933 in the nursery, the percentage of infection varying decidedly with the breeding of the wheat and also, evidently, with the time of planting.

In previous reports upon black point, durum wheats have been held to be most commonly infected (Evans [2] and Weniger)[3] although the infection has not been at all rare upon common wheats. Weniger reports that *Helminthosporium* may produce different types of infection. She mentions head blighting characterized by typical glume lesions and empty spikelets, the latter often occurring several in a group, or as individual empty spikelets (or florets) in any portion of the head. In addition, the black-point type of infection is mentioned as occurring on the seeds. Christensen [4] shows that *H. sativum* attacks different parts of the wheat plant, producing various types of lesions.

According to Christensen the various *Helminthosporium* lesions in wheat or other plants result from repeated inoculations, and Stakman holds that the disease is not systemic.[5] The lesions of black point must be induced then by local spore infection in the ovules. Evidently the time of infection, with regard to the stage of the ovule, and the circumstances attending the development of the fungus until the ripening of the seed, have not been closely studied. Henry [6]

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[1] Received for publication Feb 9, 1934; issued July 1934. Paper no. 8 of the Journal series of the North Dakota Agricultural Experiment Station.

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has shown that high temperatures are favorable for the development of *Helminthosporium*, and moisture conditions are of course important.

EXPERIMENTAL WORK

While taking notes on samples of grain threshed from individual plants, the writer was struck by the apparent differences in the size of kernels from any one plant, depending on whether the kernels showed the presence of black point or whether they were free from the infection. Certain of the plants had the weights in grams per 1,000 kernels shown in table 1.

TABLE 1.—*Weight of healthy wheat kernels and of kernels diseased with black point*

Plant no.	Weight per thousand kernels—	
	Healthy	Black-point
	Grams	*Grams*
48.12.247	27.1	33.1
48.12.257	30.7	35.9
48.12.259	26.2	33.9
48.12.288	30.2	36.1
48.12.298	32.2	38.4

The infected kernels in these instances weighed about 20 percent more than the kernels showing no infection.

Of the plants in the group studied in detail, about one half were eliminated in the field because of sterile florets. The remaining plants showed a small amount of floret sterility, but with the exception of 1 or 2 spikes, perhaps no more than might be expected in view of the extreme heat which had prevailed.

Sixteen plants were used in the study of relative weights of kernels. The kernels were carefully removed from the spike and laid in order in depressions cut in paraffin blocks and then weighed to fifths of milligrams. The 16 plants had 63 spikes and 2,030 kernels.

A cursory examination suggested that the differences in kernel weight might be ascribed to a differential incidence of black point on the different kernels of the spikelet relative to position. While the third kernel of the spikelet, when present, showed lesions less commonly than the basal kernels, it became evident that this did not entirely explain the differences.

TABLE 2.—*Number and weight of black-point and healthy kernels found paired in the spikelet, unpaired basal kernels, and kernels of the third floret*

Item	Black-point kernels		Healthy kernels		Excess weight of diseased over healthy kernels
	Number	Average weight	Number	Average weight	
		Milligrams		*Milligrams*	*Milligrams*
Paired	344	39.0±0.17	1,036	36.3±0.13	2.7±0.21
Unpaired	34	35.0± .71	191	32.5± .37	2.5± .80
Third floret	19	31.0± .63	406	27.1± .20	3.9± .66
Total or average	397	38.3± .18	1,633	33.5± .12	[a] 4.7± .22

[a] This value of 4.7 is simply the difference of the weighted means in columns 3 and 5.

The kernels, both those infected with black point and the healthy ones, were placed in three categories. Kernels found paired in the two basal florets comprised the major group; kernels of the third floret, the second group; and unpaired kernels of the basal florets, the third group. The number of kernels, and the average weight per kernel in each of the three classes, and the total weight, are given in table 2.

The elimination of the kernels found in the unpaired and in the third florets reduces the average difference in weight per kernel from 4.7 to 2.7 mg, but even this difference is highly significant as the odds are 3.3×10^{17} to 1 against the probability that a deviation as large as, or larger than, the one indicated could have arisen from random sampling. In the comparison shown in table 2, of the kernels of the third floret, the kernels of the basal spikelet which were significantly below the average in weight and were not infected, are included. Omitting the kernels of the basal spikelet, the difference in weight is still 3.7 ± 0.66 mg. The difference in weight in the unpaired basal kernels is 2.5 ± 0.8 mg and is significant. Considering the 16 plants individually, the black-point kernels of 15 of the plants were the heavier, and the difference was significant in 9 of the 15 cases. In the exceptional case the healthy kernels had the greater weight, but the difference was not significant.

STUDY OF KERNEL WEIGHT ALONG THE SPIKE

In studying the paired kernels attention was given to the disposition of the black-point and healthy kernels along the spike. The results for the 16 plants are summarized in table 3.

TABLE 3.—*Distribution along the spike of black-point and healthy kernels, in pairs, the differences in kernel weight and percentage distribution of kernel weight*

Spikelet no.	Black-point				Healthy				Differences in weight between diseased and healthy kernels		Healthy kernels
	Kernels		Kernel weight per spikelet	Distribution of kernel weight	Kernels		Kernel weight per spikelet	Distribution of kernel weight			
	Number	*Percent*	*Milligrams*	*Percent*	*Number*	*Percent*	*Milligrams*	*Percent*	*Milligrams*	*Percent*	*Percent*
1	27	7.9	35.7±0.55	7.2	145	14.0	32.9±0.30	12.7	2.9±.61	−5.5	84.4
2	52	15.1	40.1±.33	15.5	156	15.1	37.3±.26	15.5	2.8±.42	0	75.0
3	61	17.7	40.6±.33	18.5	157	15.1	39.2±.26	16.4	1.4±.42	2.1	72.1
4	75	21.8	41.0±.26	23.0	151	14.6	38.8±.27	15.6	2.2±.38	7.4	66.9
5	68	19.8	39.3±.33	19.9	148	14.3	38.0±.33	15.0	1.4±.47	4.9	68.5
6	39	11.3	35.4±.61	10.3	155	15.0	34.9±.34	14.4	.5±.70	−4.1	79.9
7	16	4.7	35.2±1.26	4.2	88	8.5	32.3±.44	7.6	2.9±1.34	−3.4	84.6
8	6	1.7	31.7±1.48	1.4	32	3.1	29.1±.77	2.5	2.6±1.67	−1.1	84.2
9	0	.0	.0	.0	4	.4	34.2	.4		−.4	100.0
Total or average	344	100.0	39.0±.17	100.0	1,036	100.1	36.2±.13	100.1	2.8±.21	−.1	

From spikelet 1 (basal) to 6 the percentage distribution of the healthy kernels approaches uniformity, varying but 1.1 percent, while the distribution of black-point kernels over the same spikelets shows a range of 13.9 percent. The higher percentages for black-point kernels are found in the center of the spike, and it is there that the heavier healthy as well as black-point kernels are found. The average weights per kernel for each spikelet are multiplied by the corresponding percentage distributions, and these results, for the two groups, reduced to percentages, are found in columns 5 and 9. When the paired kernels in the two basal florets are studied, it is found that the heavier kernels are those which carry the greater amount of black point. The net results of this difference in weight are indicated in column 10.

The middle kernels of the spike are fertilized first, but the total time for blooming was quite certainly not over 3 days, as the weather was hot and dry. This earlier fertilization may have been responsible in part for a better nutrition of the central kernels and their consequent greater weight. The fungus may have found easier access to the ovules receiving the better nutrition. The time element may have been a factor influencing the place of infection on the spike, but it is doubtful whether the entire difference in weight can be attributed to this difference in time of infection.

It is seen in table 3 that differences in weight between the black-point and healthy kernels are found regularly in each spikelet. When the probable errors were calculated the differences were found to be statistically significant in the five lower spikelets. The few black-point kernels in 2 of the 3 upper spikelets resulted in probable errors too large for the differences to be significant although the absolute differences are relatively large. The sixth spikelet showed a small difference in weight in favor of the black-point kernels. The differences in the standard deviations are very generally contrary to those of the means, in that the distributions of the healthy kernels show greater variability than do those of the black-point kernels. The coefficients of variability of the two series, black point and healthy, for the eight spikelets are shown in table 4.

TABLE 4.—*Coefficients of variability of weights of the basal pair of kernels in the two groups, black point and healthy*

Spikelet no.	Coefficient of variability			Spikelet no.	Coefficient of variability		
	Black-point kernels	Healthy kernels	Difference		Black-point kernels	Healthy kernels	Difference
1	11.70±1.11	16.44±.67	4.74±1.30	5	10.27±.60	15.93±.64	5.66±.88
2	8.66±.58	12.69±.49	4.03±.76	6	15.80±1.25	17.92±.71	2.12±1.44
3	9.32±.57	12.24±.46	2.92±.73	7	20.63±2.56	18.67±.99	−1.96±2.75
4	8.08±.45	12.46±.49	4.38±.67	8	15.42±3.36	21.90±.97	6.48±3.51

In spikelets 1 to 5 the distribution of the healthy kernels has a variability significantly greater than that of the black-point kernels. If the two series of distributions are merged so that the distribution of the total healthy can be compared with the distribution of the total black point it becomes evident where the greater variability of the healthy-kernel series originates. The two distributions expressed in percentages are shown in table 5.

The sums of the positive and negative differences are necessarily equal. The healthy kernels show an excess of variates in the lower weight classes. This is suggested by the two means (table 2). The excess of distribution of the healthy kernels extends over 11 of the lower weight and 1 of the higher weight classes, while the corresponding deficiency is confined to 6 classes in the higher ranges. Skewness is negative in both cases but more pronounced in the case of the healthy kernels. The greater variability shown in table 5 in the healthy kernels characterizes most of the spikelets and especially those which have the greater numbers of kernels.

TABLE 5.—*Percentages of healthy and black-point kernels in the various weight classes*

Item	Percentage of kernels in weight class indicated									
	16 to 17 mg	18 to 19 mg	20 to 21 mg	22 to 23 mg	24 to 25 mg	26 to 27 mg	28 to 29 mg	30 to 31 mg	32 to 33 mg	34 to 35 mg
Healthy kernels	0.4	0.6	1.2	1.5	2.4	4.0	4.1	6.7	8.0	11.1
Black-point kernels	.3	.3	0	0	.6	.6	1.7	4.6	4.1	7.6
Difference	.1	.3	1.2	1.5	1.8	3.4	2.4	2.1	3.9	3.5

Item	Percentage of kernels in weight class indicated								
	36 to 37 mg	38 to 39 mg	40 to 41 mg	42 to 43 mg	44 to 45 mg	46 to 47 mg	48 to 49 mg	50 to 51 mg	Total
Healthy kernels	12.3	13.7	15.5	9.0	6.2	2.4	0.8	0.2	100.1
Black-point kernels	11.6	18.0	20.1	15.1	10.2	4.1	.6	.6	100.1
Difference	.7	−4.3	−4.6	−6.1	−4.0	−1.7	.2	−.4	.0

A study was made of the kernel weight of the first and second kernels per spikelet of spikes with nearly or quite healthy kernels. It was not evident that the lower kernel differed in weight from the kernel standing second in position. It follows, then, that the differentiation in weight of kernel with respect to incidence of black point upon the two lower kernels per spikelet is not due to the position of the kernel in the spikelet, associated with normal weight differences.

In any plant, on an average, the black-point kernels are heavier than those evidently free from disease. In part this weight difference may be ascribed to kernel position. Certain kernels of the spike are so located that their weight is decidedly less than that of kernels differently located. There is a differential in incidence of disease with regard to these two kernel groups. Thus weight differences of healthy and diseased kernels may be ascribed to weight differential due to locality in the spike, combined with a differential of disease incidence. But beyond this, between healthy and diseased kernels, weight differences are found which are not due to location in the spike, as is shown in table 2. Finally, a comparison, or series of comparisons, is to be found in the paired kernels of the basal florets of each spikelet. In each of the eight spikelets, the black-point kernels are the heavier (table 3). In these instances, also, the heavier weight of the black-point kernels is not due to any deviation from the normal in kernel size in conjunction with differences in incidence of disease.

The question arises whether the normally larger kernels of the spike were attacked by *Helminthosporium* (and *Alternaria*) or whether, when the young kernels of a group of potentially the same mature size were attacked, such kernels were somehow stimulated to develop a larger amount of endosperm[7] than if no infection had taken place. It appears that both factors are responsible for the greater weight of the black-point kernels in the spike and in the plant.

CORRELATION RESULTS

It was possible to calculate correlation coefficients between percentages of black point and yield in the 1933 crop. In one instance a considerable number of yields were calculated from 5-foot rows. These rows were planted with F_5 selections from the cross Ceres × Hope-Florence; a single mother plant seeded one row. The stands of grain varied more or less; before harvest, notes were taken on the stand, and the yields were corrected to a uniform stand. The errors involved in this correction were thought to be less than those which would arise from uncorrected yields. Countings were made on the threshed samples of the frequency of the occurrence of black point and the percentages estimated. The weight per 1,000 kernels was also determined. The means and the standard deviations of these three characters calculated from 267 variates are shown in table 6.

TABLE 6.—*Correlations*[a] *between yield of wheat, weight of black-point kernels, and percentage of black-point kernels in the 1933 crop*

Item	Yield per acre	Weight of 1,000 kernels	Black-point kernels
	Bushels	*Grams*	*Percent*
an	34.45±0.31	37.14±0.09	21.98±0.48
ndard deviation	7.57± .22	2.06± .06	11.63± .34

[a] The 3 correlation coefficients calculated were Black point and yield, 0.22±0.04; yield and 1,000-kernel weight, 0.32±0.04; black point and 1,000-kernel weight, 0.17±0.04.

None of these coefficients is very high, but the first one, which perhaps is of greatest interest, is about five times the probable error. Evidently there is a positive relationship between the presence of the infection and the yield, as secured. When the weight per 1,000 kernels is held constant the partial correlation between yield and black point is 0.18 ± 0.04. This still shows some significance.

In the cross first mentioned in this paper it was possible to calculate correlations similar to those given, except that weight of grain per plant was used instead of yield. The coefficients are as follows:

Black point and grain yield per plant	−0.09±0.04
Yield per plant and 1,000-kernel weight	.27± .04
Black point and 1,000-kernel weight	.32± .03

When the weight per 1,000 kernels is held constant the correlation between black point and grain yield per plant becomes essentially zero. The total correlation between these characters is negative but not significant. Correlation of fairly high significance is shown between black point and 1,000-kernel weight. This is in keeping with the results shown in the earlier part of this paper.

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[7] As the endosperm in a normal kernel of wheat comprises about 85 percent of the total weight it is fair to presume that the excess weight of the diseased kernels would be distributed mainly to the endosperm. The black-point kernels appeared to be normal in shape. Any contribution to the greater weight of the black-point kernels made by the substance of the invading organism must have been negligible.

DISCUSSION

More exact studies as to the causal relationships with regard to the various external factors are greatly desired. It is known that the epidemic of black point in 1933 was associated with high temperatures and low seasonal moisture conditions. The wheats studied, started heading about June 22, and blossoming was probably well under way by June 25. A comparison of temperatures and rainfall for the last 5 days of June and the first 15 days of July are shown in table 7.

TABLE 7.—*Temperatures and precipitation for the last 5 days of June and the first 15 days of July 1933*[a]

Item	June 26-30	July 1-5	July 6-10	July 11-15	Total
Temperature	°F	°F	°F	°F	
Average daily mean	75	75	73	74	----------
Normal	67	68	67	67	----------
Excess in 1933	8	7	6	7	----------
Precipitation	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
Total in 1933	1.65	0	.81	.17	2.63
Normal	.86	.77	.47	.59	2.69

[a] Data are from the official records of the United States Weather Bureau. The normal values of precipitation were taken from the Monthly Weather Review, Vol. 58, Suppl. No. 34, p. 59, May 1930.

The daily temperatures were greatly in excess of the normal for the whole 20 days and particularly during the last 5 days of June. The season was unusually dry, but from June 26 to 30, 1.65 inches was recorded, which was decidedly in excess of the expected amount for the 5-day period. Thus for a short period, in the early life of the wheat seed, conditions of high temperature and high rainfall obtained. It is quite unknown, of course, whether the weather conditions varied in such a manner during the period of infection as to account for the differences in incidence of black point which were shown to exist between basal kernels and kernels of the third floret and also between the mid-spike kernels and those near the base and near the tip of the spike. It is not conceivable that any differentiation in weight due to position could be brought about within a single spikelet or in the third-floret kernels within the spike, as was the case in several instances.

On the other hand there seems to be no report in the literature that a seed infected by a fungus is stimulated, and consequently increases in growth. If stimulation resulted in this case, an increased amount of endosperm must have been laid down, but there seems to be little or no information as to any causal relationship which might have brought this about.

SUMMARY

A heavy infection of black point, caused largely by *Helminthosporium sativum* Pam., King, and Bak., was found on various common wheats at Fargo in 1933. On any one plant, in the hybrids studied, the black-point kernels generally were definitely heavier than the kernels showing no evidences of infection.

This difference in weight can be ascribed in part to a difference in infection of kernels differing normally in size because of position in the spike. Third-floret kernels and end-spike kernels carried less infection than the heavier mid-spike kernels.

Within any kernel group of the spike, such as the third-floret group, the black-point kernels were significantly heavier than the noninfected kernels. The obvious conclusion seems to be that a portion of the weight differences results from a stimulation of the development of the endosperm following the entrance of the fungus into the developing ovule.

In one experiment, a coefficient of correlation of 0.22 ± 0.04 was found between the percentage of black point and the yield in 5-foot rows and a coefficient of 0.32 ± 0.04 was found between black point and weight of 1,000 kernels. In a study of individual plants resulting from a different cross, a correlation of −0.09 ± 0.04 was secured between the percentage of black point and the weight of the grain per plant and a correlation of 0.32 ± 0.03 was obtained between percentage of black point and the weight per 1,000 kernels.

A STATISTICAL STUDY OF THE RELATIONSHIPS BETWEEN THE CONSTITUENTS OF MILK[1]

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INTRODUCTION

The quantitative relationships of the fat, protein, total solids, and solids not fat in cow's milk have been extensively investigated by Overman, Sanmann, and Wright (*13*),[2] Gaines (*6*), Cranfield, Griffiths, and Ling (*4*, *5*), and others. The purpose of this study is to contribute further to the subject in general, but especially to extend the consideration into the field of the inorganic constituents, which have received comparatively little attention.

Many studies of the relations existing between the different constituents of milk have been undertaken from the commercial point of view, especially with reference to the detection of adulteration, the establishment of legal standards, and the enrichment of milk, particularly in fat; and other studies have been made on the origin of the various constituents of milk, the methods of their secretion, the factors affecting their elaboration, and the interrelationship of these constituents. In the present study, which is statistical in method, the immediate objective has been to discover relationships of constituents which might subsequently be subjected to intensive investigation from the physiological point of view.

The data on which this paper is based were obtained from analyses of the milk of 12 Holstein-Friesian cows during an entire period of lactation. The animals were maintained under uniform conditions of care and feeding, without pasture, and were kept indoors, except for short daily periods of controlled exercise.

In relation to feeding treatment the cows were divided into two groups, one of which received timothy hay, corn silage, and concentrate mixture; the other, alfalfa hay, corn silage, and concentrate mixture. The protein content of the rations of the two groups was approximately equalized by varying the composition of the concentrates. Within both of the groups 2 cows were given a supplement of pulverized limestone, 2 a supplement of commercial bone meal, while 2 received no mineral supplement.

No evidence was found that the composition of the rations affected the composition of the milk in any characteristic manner. The analyses of the milk, therefore, were combined into a single group for the present study.

The usual seasonal variation in the composition of milk, dependent on changes in the ration, was not brought out by the method employed in this investigation, but the changes in the composition of milk which occur with the progress of lactation were observed, and these contributed to the statistical constants determined.

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[1] Received for publication Feb. 21, 1934; issued July 1934. Published as Technical Paper no. 638 of the Institute of Animal Nutrition.

[2] Reference is made by number (italic) to Literature Cited, p. 1032.

The relationships reported are those of fat, energy, protein, total solids, solids not fat, lactose, ash, calcium, phosphorus, magnesium, chlorine, sodium, and potassium.

EXPERIMENTAL METHODS

The data treated represent 134 samples of milk which were, in greater part, composites of aliquots of each milking for 28-day periods. The time of calving, or of the end of the period of lactation, however, made certain of the milk-sampling periods shorter than the usual 28 days.

The method of preserving the milk, and the methods and results of the determinations of energy, fat, protein, total solids, and solids not fat, have been reported in a previous publication by Kahlenberg and Voris (*10*).

Ash, sodium, and potassium were determined by the official methods of the Association of Official Agricultural Chemists (*2*).

Calcium and magnesium were determined by the McCrudden method (*11, 12*), combined with the filtration and titration technic suggested by Halverson and Schulz (*8*).

Phosphorus was determined gravimetrically by the Neumann-Pemberton wet combustion method (*15*).

Chlorine was determined by the official method of the Association of Official Agricultural Chemists (*2*) except that 0.05 normal silver nitrate solution and 0.03 normal potassium thiocyanate were used instead of 0.1 normal silver nitrate and potassium thiocyanate as specified.

The percentage of lactose was obtained by subtracting the sum of the percentages of ash and protein from the percentage of solids not fat.

The statistical methods were, in general, standard procedures, in the form used by Harris and Benedict (*9*), with supplementary use of Pearson's tables (*14*).

PRESENTATION OF DATA AND DISCUSSION OF RESULTS

Table 1 summarizes the analytical data arranged in the order of increasing percentage of fat, by giving the mean percentage of each constituent corresponding to intervals of 0.2 percent in the mean fat content. The means, standard deviations, and coefficients of variation, with their respective probable errors, are given in table 2; the coefficients of correlation between the milk constituents are recorded in table 3; and the correlation coefficients are arranged with respect to magnitude and statistical significance, in the form of a frequency distribution, in figure 1.

VARIABILITY

It has been generally conceded in discussions of the composition of milk that fat is one of the most variable, if not the most variable, constituent. However, the individual inorganic constituents have not generally been taken into account—the ash usually being considered as a single ingredient—and the samples of milk have not usually been obtained from animals under conditions of experimental control.

The coefficients of variation in table 2 show that the sodium, the chlorine, and the magnesium content of milk varies much more than does the fat, and that protein varies as much as does fat. It may be that the fat content of milk is more easily affected by disturbing conditions than is the protein content, and that under the uniform conditions maintained throughout this experiment fat was less variable than it is under ordinary feeding practices.

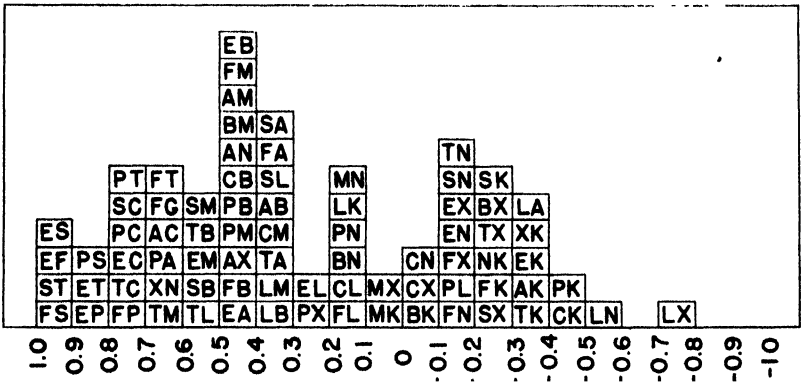

FIGURE 1.—Distribution of correlation coefficients between quantities of milk constituents *F*, fat, *E*, energy; *P*, protein, *S*, total solids, *T*, solids not fat, *L*, lactose, *A*, ash; *C*, calcium; *B*, phosphorus; *M*, magnesium; *X*, chlorine, *N*, sodium, and *K*, potassium.

TABLE 1.—*Summary of analytical data, giving average percentages of each milk constituent when mean fat content is aranged in 0.2 percent classes*

Fat range (percent)	Samples	Fat	Nitrogen	Protein	Total solids	Solids not fat	Energy per gram	Lactose
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Calories*	*Percent*
2.60 to 2.79	6	2.70	0.424	2.71	10.85	8.15	586	4.71
2.80 to 2.99	13	2.93	.434	2.77	11.37	8.44	615	4.93
3.00 to 3.19	26	3.10	.447	2.85	11.79	8.69	643	5.12
3.20 to 3.39	34	3.29	.470	3.00	12.08	8.79	666	5.06
3.40 to 3.59	13	3.46	.498	3.25	12.46	9.00	690	5.01
3.60 to 3.79	18	3.72	.510	3.31	12.68	8.96	716	4.93
3.80 to 3.99	10	3.89	.513	3.27	12.98	9.09	732	5.05
4.00 to 4.19	7	4.09	.569	3.63	13.49	9.40	774	5.01
4.20 to 4.39	3	4.25	.571	3.65	13.93	9.68	793	5.24
4.40 to 4.60	4	4.50	.600	3.83	14.40	9.90	825	5.26

Fat range (percent)	Samples	Ash	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Chlorine
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
2.60 to 2.79	6	0.742	0.081	0.143	0.094	0.013	0.085	0.132
2.80 to 2.99	13	.741	.063	.146	.103	.012	.091	.110
3.00 to 3.19	26	.737	.054	.149	.106	.012	.093	.100
3.20 to 3.39	34	.743	.055	.143	.109	.013	.094	.108
3.40 to 3.59	13	.770	.076	.140	.116	.013	.097	.117
3.60 to 3.79	18	.775	.058	.128	.121	.013	.097	.109
3.80 to 3.99	10	.768	.068	.144	.115	.015	.101	.098
4.00 to 4.19	7	.768	.059	.126	.123	.014	.095	.110
4.20 to 4.39	3	.790	.082	.121	.130	.016	.105	.109
4.40 to 4.60	4	.810	.066	.139	.128	.019	.111	.095

Among the individual inorganic constituents, sodium seems to be the most variable. This may be only apparent, the variability of the data being due in part to difficulties encountered in analysis; but chlorine, with which sodium shows the closest correlation, and which is not particularly difficult to determine, also shows a variability strikingly greater than that of any of the other constituents except magnesium. Therefore, inasmuch as sodium and chlorine are largely in combination with each other, sodium chloride is presumably the most variable constituent of milk. Possible explanations of this variability are suggested in the discussion of the correlation coefficients.

TABLE 2.—*Means, standard deviations, and coefficients of variation of constituents of milk*

Constituent	Means	Standard deviations	Coefficients of variation
Fat	3.4095±0.0245	0.4207±0.0173	12.34±0.52
Protein	3.0770± .0224	.3838± .0158	12.47± .52
Total solids	12.2713± .0481	.8250± .0340	6.72± .28
Solids not fat	8.8619± .0277	.4752± .0196	5.36± .22
Energy	680.075 ±3.299	56.61 ±2.332	8.32± .35
Lactose	5.0274± .0181	.3108± .0128	6.18± .26
Ash	.7547± .0024	.0420± .0017	5.57± .23
Sodium	.0576± .0011	.0184± .0008	31.94±1.44
Potassium	.1392± .0010	.0172± .0007	12.36± .52
Calcium	.1117± .0007	.0117± .0005	10.47± .44
Magnesium	.0131± .0002	.0030± .0001	22.90± .99
Phosphorus	.0951± .0005	.0090± .0004	9.46± .39
Chlorine	.1077± .0013	.0216± .0009	20.06± .86

TABLE 3.—*Coefficients of correlation between quantities of milk constituents*

Constituent	Correlation r
Fat and—	
Energy	0.9502±0.0057
Total solids	.9076± .0103
Protein	.7000± .0297
Solids not fat	.6885± .0306
Calcium	.6646± .0325
Magnesium	.4690± .0455
Phosphorus	.4166± .0482
Ash	.3823± .0498
Lactose	.1264± .0573
Sodium	−.1027± .0576
Chlorine	−.1194± .0574
Potassium	−.2242± .0553
Energy and—	
Total solids	.9817± .0021
Fat	.9502± .0057
Solids not fat	.8565± .0155
Protein	.8101± .0200
Calcium	.7698± .0237
Magnesium	.5612± .0399
Phosphorus	.4787± .0449
Ash	.4125± .0484
Lactose	.2491± .0547
Sodium	−.1201± .0574
Chlorine	−.1452± .0570
Potassium	−.3252± .0521
Protein and—	
Total solids	.8760± .0136
Energy	.8101± .0200
Solids not fat	.7868± .0222
Calcium	.7728± .0235
Fat	.7000± .0297
Ash	.6194± .0359
Phosphorus	.4354± .0472
Magnesium	.4262± .0477
Chlorine	.2197± .0555
Sodium	.1445± .0571
Lactose	−.1134± .0575
Potassium	−.4934± .0441
Total solids and—	
Energy	.9817± .0021
Solids not fat	.9382± .0070
Fat	.9076± .0103
Protein	.8760± .0136
Calcium	.7798± .0229
Magnesium	.5893± .0380
Total solids and—Continued	
Phosphorus	0.5448±0.0410
Ash	.3843± .0497
Lactose	.3667± .0504
Sodium	−.1555± .0569
Chlorine	−.2064± .0558
Potassium	−.2932± .0533
Solids not fat and—	
Total solids	.9382± .0070
Energy	.8565± .0155
Protein	.7868± .0222
Calcium	.7670± .0240
Fat	.6885± .0306
Magnesium	.6083± .0367
Phosphorus	.5813± .0386
Lactose	.5320± .0418
Ash	.3362± .0517
Sodium	−.1777± .0564
Chlorine	−.2506± .0546
Potassium	−.3061± .0528
Lactose and—	
Solids not fat	.5320± .0418
Total solids	.3667± .0504
Magnesium	.3281± .0520
Phosphorus	.3005± .0530
Energy	.2491± .0547
Potassium	.1767± .0565
Calcium	.1306± .0573
Fat	.1264± .0573
Protein	−.1134± .0575
Ash	−.3891± .0494
Sodium	−.5007± .0437
Chlorine	−.7030± .0295
Ash and—	
Calcium	.06378± .0346
Protein	.6194± .0359
Magnesium	.4579± .0460
Sodium	.4511± .0464
Chlorine	.4485± .0481
Energy	.4125± .0484
Total solids	.3843± .0497
Fat	.3823± .0498
Phosphorus	.3564± .0509
Solids not fat	.3362± .0517
Potassium	−.3219± .0522
Lactose	−.3891± .0494

TABLE 3.—*Coefficients of correlation between quantities of milk constituents*—Con.

Constituent	Correlation *r*	Constituent	Correlation *r*
Calcium and—		Chlorine and—	
Total solids	0.7793±0.0229	Sodium	0.6109±0.0365
Protein	.7728± .0235	Ash	.4185± .0481
Energy	.7698± .0237	Protein	.2197± .0555
Solids not fat	.7670± .0240	Magnesium	.0444± .0582
Fat	.6646± .0325	Calcium	−.0620± .0580
Ash	.6378± .0346	Fat	−.1194± .0574
Phosphorus	.4508± .0464	Energy	−.1452± .0570
Magnesium	.3365± .0517	Total solids	−.2064± .0558
Lactose	.1306± .0573	Solids not fat	−.2506± .0546
Chlorine	−.0620± .0580	Phosphorus	−.2632± .0542
Sodium	−.0785± .0579	Potassium	−.3825± .0497
Potassium	−.4399± .0470	Lactose	−.7030± .0295
Phosphorus and—		Sodium and—	
Solids not fat	.5813± .0386	Chlorine	.6109± .0365
Total solids	.5448± .0410	Ash	.4511± .0464
Energy	.4787± .0449	Magnesium	.1926± .0561
Magnesium	.4519± .0464	Protein	.1445± .0571
Calcium	.4508± .0464	Phosphorus	.1383± .0572
Protein	.4351± .0472	Calcium	−.0785± .0579
Fat	.4166± .0482	Fat	−.1027± .0576
Ash	.3564± .0509	Energy	−.1201± .0574
Lactose	.3005± .0530	Total solids	−.1555± .0569
Sodium	.1383± .0572	Solids not fat	−.1777± .0564
Potassium	−.0095± .0583	Potassium	−.2367± .0550
Chlorine	−.2632± .0542	Lactose	−.5007± .0437
Magnesium and—		Potassium and	
Solids not fat	.6083± .0367	Lactose	.1767± .0565
Total solids	.5893± .0380	Magnesium	.0382± .0582
Energy	.5612± .0399	Phosphorus	−.0095± .0583
Fat	.4690± .0455	Fat	−.2242± .0553
Ash	.4579± .0460	Sodium	−.2367± .0550
Phosphorus	.4519± .0464	Total solids	−.2932± .0533
Protein	.4262± .0477	Solids not fat	−.3061± .0528
Calcium	.3365± .0517	Ash	−.3219± .0522
Lactose	.3281± .0520	Energy	−.3252± .0521
Sodium	.1926± .0561	Chlorine	−.3825± .0497
Chlorine	.0444± .0582	Calcium	−.4399± .0470
Potassium	.0382± .0582	Protein	−.4934± .0441

The exceptional variability of magnesium which was observed has also been noted by Allen (*1*), but no adequate explanation of this fluctuation is apparent.

Solids not fat show the least variability, followed, in the order of increasing variability, by ash, lactose, total solids, energy, phosphorus, calcium, chlorine, magnesium, and sodium.

CORRELATIONS

In the interpretation of the correlation coefficients any correlations greater than 0.4600, that is, correlations of a magnitude greater than 10 times their probable error, have been considered of definite significance. Correlations of from 0.3130 to 0.4600, which are more than 6 and less than 10 times their probable error, have been designated as of doubtful significance, and correlations of less than 6 times their probable error have not been considered significant.

The following general statements of significant relationships were observed:

(1) As the percentage of fat increases, the energy, and the percentages of total solids, protein, solids not fat, calcium, and magnesium increase.

(2) As the energy value increases, the percentages of total solids, solids not fat, protein, calcium, magnesium, and phosphorus increase.

(3) As the percentage of protein increases, the percentages of total solids, solids not fat, calcium, and ash increase, and the percentage of potassium decreases.

(4) As the percentage of total solids increases, the percentages of solids not fat, calcium, magnesium, and phosphorus increase.

(5) As the percentage of solids not fat increases, the percentages of calcium, magnesium, phosphorus, and lactose increase.

(6) As the percentage of lactose increases, the percentages of sodium and chlorine decrease.

(7) As the percentage of ash increases, the percentage of calcium increases.

(8) As the percentage of chlorine increases, the percentage of sodium increases.

Among the correlations of doubtful significance it is of interest to note that as magnesium increases the percentages of ash, phosphorus, protein, calcium, and lactose increase. It has been noted above that magnesium is significantly correlated with solids not fat, total solids, energy, and fat. Thus, in 9 of the 12 comparisons, magnesium shows a significant, or probably significant, positive correlation. Similarly, calcium shows high positive correlations with total solids, protein, energy, solids not fat, fat, and ash, with possibly significant positive correlations with phosphorus and magnesium.

Among the negative correlations of special interest are those of potassium with protein and calcium; and of lactose, which is a relatively constant constituent, with sodium and chlorine, which are extremely variable. The findings with reference to lactose, sodium, and chlorine are in harmony with the conclusion of Porcher (*16*) that milk with high lactose content is usually low in sodium chloride, and that mainly by variation in the sodium chloride in the milk it is maintained isotonic with the blood.

Gowen and Tobey (*7*) state that the osmotic pressure of milk is very largely determined by the lactose content, whereas the osmotic pressure of blood is relatively little determined by its sugar content but mostly by its salt content, referring to six major osmotically active elements, potassium, sodium, calcium, magnesium, phosphorus, and chlorine. In the maintenance of the isotonicity of blood and milk, the milk of a lower lactose content would have to increase its content of salts in a rather pronounced degree to make up for this deficiency of lactose. Gowen and Tobey propose to compute the osmotic pressure from the millimolar concentration of lactose and ash found in the milk. From the present data lactose does reveal a negative correlation with ash, but among the individual elements only sodium and chlorine were found negatively correlated with lactose.

Blackwood and Stirling (*3*) consider that by far the largest part of any osmotic change taking place during milk secretion is to be accounted for by the synthesis of protein and carbohydrate from glucose and amino acids. They consider that during milk secretion a transudate is separated from the blood, having the same osmotic pressure as blood, and that during synthesis water is returned to the blood to maintain the tonicity of the cell fluid.

In the present study protein shows no significant correlations, positive or negative, with lactose and sodium chloride; and these correlations, as contrasted with the significantly negative correlation of lactose and sodium chloride, suggest a dissimilar relation of protein and lactose in the maintenance of the isotonicity of the blood and milk.

The narrow range of variation in the hydrogen-ion concentration of freshly drawn milk, between 6.50 and 6.65, is probably maintained chiefly through the phosphates and casein present. It was shown by Rice and Markley (*17*) that the hydrogen-ion concentration of milk of high acidity, as determined by titration, is affected by the addition of acid or alkali to a much lesser extent than is the hydrogen-ion concentration of milk with low acidity. Their explanation is that in high-acid milk the phosphate and casein are present in greater quantities than in low-acid milk, and that the buffer action is greater in the former case than in the latter. The negative correlation of potassium and protein is in harmony with this conclusion, and since calcium is largely associated with protein the negative correlation of potassium and calcium seems to be a part of the same picture. However, there is no correlation, either positive or negative, between potassium and phosphorus. In fact, potassium shows no significant positive correlation with any of the constituents determined, though it may be correlated with undetermined constituents, for instance, with citric acid.

Wright (*18*) has shown experimentally that $CaHPO_4$ in solutions of calcium caseinate may be held in stable colloidal solutions up to the concentrations of calcium and phosphorus found in milk. He suggests that the calcium and phosphorus which diffuse from the blood into the mammary gland may thus be held in colloidal combination by the casein in the milk cells. Alkali renders this combination unstable, with which fact the negative correlations of potassium with protein and calcium, as found in this study, seem to be in harmony. This calls attention to the slight degree of correlation found between potassium and phosphorus, and suggests that phosphorus is associated with other constituents than calcium and casein—which is borne out by the fact that the ratio of phosphorus to calcium in calcium caseinate is 0.8, and in $CaHPO_4$ it is 0.775, whereas the ratio between the average percentages of phosphorus to calcium found in milk in the present study is 0.851.

In consideration of the questionable significance of correlations as evidence however, no effort will be made to interpret these observations closely. Correlations can only suggest, and the facts as to origin and relationships of the constituents of milk must be established by evidence of more direct and less equivocal character.

SUMMARY

Statistical data based on the analyses of 134 samples of Holstein-Friesian milk representing one entire lactation period for each of 12 cows are reported. The analyses include fat, energy, protein, total solids, solids not fat, lactose, ash, calcium, phosphorus, magnesium, chlorine, sodium, and potassium. The means, standard deviations, and coefficients of variation of each constituent with their corresponding probable errors, are reported, and the coefficients of correlation of each constituent with every other constituent are given.

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INFLUENCE OF CALCIUM PHOSPHORUS-INTAKE ON BOVINE BLOOD[1]

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INTRODUCTION

Phosphorus is present in every cell and fluid of the body. It is vitally concerned with carbohydrate, fat, and some phases of protein metabolism. It is essential in muscular contractions and in the functioning of the central nervous system. It enters into the buffering powers of the blood and other tissues. Phosphorus, together with calcium, plays a fundamental role in bone formation. It is, then, not surprising to find profound physiological and anatomical disturbances occurring in animals on a phosphorus-deficient diet. In cattle such a deficiency may manifest itself by hypophosphoraemia, osteophagia, and osteoporosis, and by osteomalacia in the adult (*15*).[2] In the early stages the consumption of food is usually adversely affected (*26*). On phosphorus-deficient rations the milk yield is usually low (*2*) and there is a marked inhibition of oestrum (*4*). Usually before most of the symptoms appear, hypophosphoraemia manifests itself (*3*, *24*).

Aphosphorosis of cattle is known to occur in many regions (*5*, *25*). It is probably even more widely spread than is known at the present time, where the following conditions occur: The forage is produced on phosphorus-deficient soil and consequently is low in phosphorus; the cattle are kept on too restricted rations, the main constituents of which are deficient in phosphorus; the intake of calcium is excessive in relation to the intake of phosphorus; and the phosphorus requirements are extremely high and hence not met by the ordinary rations, as in the case of the lactating cow.

It has been suggested that the estimation of inorganic blood phosphorus gives an early diagnosis of aphosphorosis and helps to differentiate it from picas having a different origin (*14*, *26*). With sufficient information on the optimum inorganic phosphorus of the blood under varying conditions, it appears probable that blood analyses may be used to discover those animals which are on phosphorus-deficient diets, and hence the proper phosphorus supplement may be prescribed. In this manner, at least in a degree, it may be possible to prevent the modern dairy cow from passing through a mild form of osteoporosis at each lactation. This is an extreme instance and at times may not be due to a deficient ration but rather to an overdeveloped udder outrunning the absorptive capacity of the gut for phosphorus (*6*). However, Huffman and his coworkers (*12*) found that even high-yielding cows (2,000 gallons) can be maintained in mineral equilibrium (positive calcium balance) upon ordinary mineral-rich rations. Nevertheless, the margin is often exiguous, and little may be required to change a positive balance to a negative one.

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[2] Reference is made by number (italic) to Literature Cited, p. 1040.

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EXPERIMENTAL ANIMALS AND PROCEDURE

Data reported in this paper represent blood analyses obtained from 40 grade yearling beef steers, averaging 585 pounds in weight and of similar breeding and previous treatment. They were carefully sorted into five groups as nearly alike as possible as to weight, quality, and condition. They were fed for a period of 150 days on the following rations:

Lot 1, pressed beet pulp, beet molasses, alfalfa hay, and salt.
Lot 2, pressed beet pulp, beet molasses, alfalfa hay, and cottonseed cake.
Lot 3, pressed beet pulp, beet molasses, alfalfa hay, and steamed bone meal.
Lot 4, pressed beet pulp, beet molasses, alfalfa hay, and mill-run bran.
Lot 5, pressed beet pulp, beet molasses, alfalfa hay, and ground barley.

The average daily ration consumed by each lot, the daily calcium and phosphorus intakes, and the calcium-phosphorus ratio in the rations are shown in table 1.

Lot 1, receiving beet pulp, molasses, alfalfa, and salt, obtained less than 50 percent of the phosphorus received by those on the ration supplemented with cottonseed cake. The phosphorus intake of lot 5, where barley was given, was also low; that of the remaining lots was high. There were also considerably greater quantities of calcium in the rations of the animals receiving phosphorus supplements. This is especially noticeable in the lot receiving cottonseed cake. The calcium oxide intake in the ration of lot 1 equaled 0.74 percent; of lot 2, 0.69 percent; of lot 3, 0.48 percent; of lot 4, 0.52 percent; and of lot 5, 0.73 percent. All these animals received rations containing more than 0.45 percent of calcium oxide placed as the minimum by the Wisconsin workers (*10*) for dry dairy cows. Phosphorus was low in the rations of lots 1 and 5 when compared with the quantities recommended by Kellner (*13*) and Armsby (*1*).

The animals receiving no phosphorus supplements were getting considerably less than the 30 g of phosphorus pentoxide per day, which Theiler, Green, and Du Toit (*27*) laid down as the minimum for heifers. Where barley was the supplement, the animals were receiving 30 g; all others were receiving considerably more than the minimum requirements.

Theiler, Green, and Du Toit (*26*) conclude that inasmuch as minimum requirements of growth are higher in the case of phosphorus than in the case of calcium, a ratio of calcium oxide to phosphorus pentoxide as high as 3 to 1 is not necessarily disadvantageous. Lots 2 and 3 were receiving rations in which the ratio of calcium oxide to phosphorus pentoxide was 3 to 1. Lot 1 received calcium oxide to phosphorus pentoxide in a ratio of 4.6 to 1 and lot 5 in the ratio of 4.1 to 1. It is evident, therefore, that in lots 1 and 5 phosphorus could have been profitably added. One is prone to ask: Could greater quantities of phosphorus have been added advantageously to the rations of lots 2, 3, and 4? Theiler, Green, and Du Toit (*26*) state that when calcium is low it is probable that a relatively high proportion of phosphorus may facilitate the absorption of calcium and thus reduce the risk of calcium starvation. When phosphorus is low, a relatively high calcium intake may reduce the absorption of phosphorus and in this way increase the danger of aphosphorosis.

TABLE 1.—*Average daily ration consumed, average daily intake of calcium and phosphorus, and the calcium-phosphorus ratio in the rations*

Lot no.	1		2		3		4		5	
Average ration consumed ... pounds	Beet pulp	21.4	Cottonseed cake	1.8	Bone meal	0.1	Mill-run bran	2.7	Ground barley	3.6
	Molasses	2.5	Beet pulp	34.6	Beet pulp	48.6	Beet pulp	42.0	Beet pulp	24.7
	Alfalfa	7.1	Molasses	2.5	Molasses	2.5	Molasses	2.5	Molasses	2.5
	Salt	.09	Alfalfa	6.4	Alfalfa	6.3	Alfalfa	6.3	Alfalfa	6.7
			Salt	.06	Salt	.04	Salt	.04	Salt	.04
Phosphorus as P_2O_5 in ration ... grams		23		48		41		48		30
Calcium as CaO in ration ... do		105		143		127		127		125
$\frac{CaO}{P_2O_5}$ ... ratio		4.6		3.0		3.1		2.6		4.1

Samples of blood were collected in the morning before feeding to obviate the action of recently ingested carbohydrates on blood inorganic phosphorus. It was found in preliminary tests in 1932, when this precaution was not taken, that the blood inorganic phosphorus often fell to an extremely low level, probably due to the large quantities of soluble carbohydrates consumed.

The blood was collected from the jugular vein in two 4-ounce bottles. The sample for phosphorus determination was quickly mixed with sodium oxalate, immediately taken to the laboratory, and the plasma centrifuged out. The phosphorus was determined in the plasma by the Youngberg method (*11*); the calcium in the serum of the second sample by the Clark-Collip modification of the Krammer-Tisdal method (*11*). Results are reported as milligrams of phosphorus in 100 cc of blood plasma and milligrams calcium in 100 cc of blood serum (table 2). Samples were taken at the beginning of the experiment and during each succeeding month. This work represents analyses of individual samples and not the composite of three samples taken on successive days as reported by some workers. In this work, however, samples were taken from each of eight animals receiving the same treatment. This should eliminate, to a degree, individual and daily fluctuations in the calcium and phosphorus content of the blood.

EXPERIMENTAL DATA

The average calcium content of the blood of the various lots on January 21, 1933, varied from 12.25 mg per 100 cc of blood serum to 13.16 mg. With few exceptions, there is only a small variation within the different groups (table 2).

TABLE 2.—*Milligrams of calcium per 100 cc of blood serum and of inorganic phosphorus per 100 cc of blood plasma in blood of steers fed different supplements to the ration, Jan. 21 and Feb. 28, 1933*

DATA OF JAN. 21, 1933

Steer no.	Lot 1, no supplement		Lot 2, cottonseed cake			Lot 3, steamed bone meal			Lot 4, mill-run bran			Lot 5, ground barley		
	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P
3	13.50	2.87	1	12.00	2.30	2	12.00	1.89	7	13.50	2.56	5	13.25	2.52
12	12.00	2.71	6	13.00	2.18	4	11.25	2.68	13	12.00	3.16	8	11.75	3.76
22	14.00	3.07	10	11.75	2.47	16	13.50	3.65	21	12.50	2.72	23	12.00	2.78
29	13.75	2.26	15	13.75	2.15	18	13.50	3.40	24	14.00	1.74	28	13.25	2.01
40	13.75	2.54	25	14.00	1.67	32	11.00	3.51	26	11.25	2.93	31	12.50	3.46
41	12.00	2.59	30	13.00	3.10	33	11.25	2.75	34	11.75	2.38	36	10.50	2.96
46	11.50	2.79	42	13.75	2.21	14	12.00	2.47	35	14.00	1.69	44	13.00	3.21
49	13.75	2.24	43	13.75	3.23	48	16.00	2.72	45	13.75	3.73	50	11.75	2.49
Average.	13.04	2.63		13.13	2.41		12.56	2.88		12.84	2.61		12.25	3.01

DATA OF FEB. 28, 1933

Steer no.	Lot 1, no supplement		Lot 2, cottonseed cake			Lot 3, steamed bone meal			Lot 4, mill-run bran			Lot 5, ground barley		
	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P	Steer no.	Ca	P
3	13.3	3.13	1	13.5	4.49	14	13.3	4.74	21	14.0	6.67	50	15.8	3.38
12	13.0	3.25	25	14.3	4.07	48	13.5	6.25	45	14.0	4.55	44	14.0	4.35
22	13.0	4.13	6	13.8	3.54	2	12.8	6.29	34	14.8	4.52	5	[illegible]	3.02
29	15.0	3.13	43	13.5	4.55	16	12.8	5.71	13	14.0	7.04	23	14.5	3.37
40	13.5	3.73	15	14.0	4.07	18	14.5	4.17	24	14.5	4.39	8	14.8	4.42
41	12.8	3.70	42	14.3	5.38	33	14.3	5.38	26	11.3	5.95	36	15.3	3.31
46	14.0	3.91	10	13.0	4.39	32	13.0	6.58	35	14.5	3.57	28	14.0	3.16
49	13.0	3.85	30	14.0	4.52	4	13.5	5.65	7	14.0	6.62	31	15.3	4.35
Average	13.4	3.60		13.8	4.38		13.5	5.60		13.9	5.41		14.9	3.67

These animals had grazed on the range plants which were grown on the highly calcareous soils of Idaho and consequently were high in calcium. This may account for the high calcium and comparatively low phosphorus content of the animal's blood.

The inorganic phosphorus of the blood varied from 2.41 to 3.01 mg per 100 cc of blood plasma. These results are low when compared with those obtained by Eckles and his coworkers (*5*) on animals receiving sufficient phosphorus and point strongly to the conclusions that all these animals, although on a ration high in alfalfa hay grown on phosphorus-rich soils, would have benefited if given a phosphorus supplement.

The phosphorus-calcium ratio in the blood varied from 1 to 4 in barley-fed steers and from 1 to 5.5 in animals receiving cottonseed cake.

On February 28, 1933, the blood was again analyzed (table 2). A slight increase in the blood calcium had occurred. Even where the animals received the calcium supplements there was an increase of only 1 mg per 100 cc of blood serum, which is in keeping with the findings of Theiler, Green, and Du Toit (*26*). The phosphorus, however, had markedly increased. The animals receiving bone meal and mill-run bran yielded blood phosphorus well above 5 mg per 100 cc of blood plasma. In those animals receiving no phosphorus supplement and those receiving ground barley, the blood phosphorus was lower, being 3.60 and 3.67 mg per 100 cc of blood plasma, respectively. The phosphorus-calcium ratio, while still varying widely in the blood of different lots, was much narrower.

Blood samples were also taken on April 1, April 27, and June 1, 1933. These showed the same regularity in each group, as did the analyses made on January 21 and February 28. Hence, only the averages of these determinations are given (table 3). Each reported result represents the averages of the analyses made on eight different animals receiving the same ration which should rule out individual differences that occur when averages are made from individual animals.

TABLE 3.—*Average milligrams of calcium in 100 cc of blood serum and of inorganic phosphorus per 100 cc of blood plasma in the blood of steers fed different supplements to the ration on given dates*

[Averages represent 8 determinations made on different animals receiving the same rations]

CALCIUM

Date of sampling	Lot 1, no supplement	Lot 2, cottonseed cake	Lot 3, steamed bone meal	Lot 4, mill-run bran	Lot 5, ground barley
1933					
Jan. 21	13.16	13.13	12.58	12.84	12.25
Feb. 28	13.40	13.18	13.50	13.90	14.90
Apr. 1	15.22	12.03	12.31	12.63	12.56
Apr. 27	13.41	12.19	12.19	11.88	12.59
June 1	14.10	13.00	13.00	12.20	12.80
INORGANIC PHOSPHORUS					
1933					
Jan. 21	2.63	2.41	2.88	2.61	3.01
Feb. 28	3.60	4.38	5.60	5.41	3.67
Apr. 1	2.67	4.12	5.22	4.52	3.58
Apr. 27	2.60	4.28	4.71	4.52	3.35
June 1	3.11	4.45	4.35	4.98	3.73

When placed on the experimental ration, these cattle were taken from feeds high in calcium. Moreover, all experimental rations carried calcium well above the minimum requirements for such animals; consequently, their blood calcium showed no appreciable variation that could be ascribed to these rations. The greatest variation is shown in the lot receiving no phosphorus supplement. Due to the drop in phosphorus, nature may have attempted to maintain an approximate uniform calcium times phosphorus concentration. Palmer and Eckles (*20*) produced hypercalcemia and hypophosphoraemia in cows by feeding diets low in phosphorus. Had the animals used in the experiments here reported been younger, it is highly probable that greater variations would have occurred in the serum calcium, for it is not easy to raise the concentration of serum calcium far above normal by feeding calcium salts or high calcium-containing feed; it is even more difficult to lower the serum calcium of normal full-grown animals by altering the intake of calcium (*23, v. 1, p. 814*).

The inorganic phosphorus of the blood plasma on January 21, when the cattle were placed on the various rations, was low. There was an increase on February 28, greatest in the case of steers receiving bone meal and least where no phosphorus supplement was given. The concentrations remained quite uniform throughout the remainder of the experiment. Only occasionally did the inorganic phosphorus content reach 5-mg concentration, making it highly probable that all of these animals would have benefitted by an increase in the phosphorus supplement due to the high calcium intake.

DISCUSSION

It has been stated (*23*) that calcium and phosphorus in the circulating plasma, other factors remaining constant, bear a reciprocal relationship to one another. Harnes (*8, 9*) tested this biometrically on the blood of rabbits and found a small negative correlation between the calcium and inorganic phosphorus. Palmer and his coworkers (21) found no correlation between calcium and inorganic phosphorus and consider Harnes' coefficient so small in relation to the probable error as to be mathematically insignificant. Calculations have been made of the correlation coefficients between calcium and phosphorus in the 199 analyses, as reported herein. The formulas given by Pearl (*22*) were used: $r_{12}=\frac{(x_1x_2)}{N\sigma_1\sigma_2}$ and $P.E._r=0.67449\,\frac{1-r^2}{\sqrt{N}}$.

The values for r_{12} on the following dates are:

Jan. 21, -0.1660 ± 0.1037 ($n=40$)
Feb. 28, -0.3333 ± 0.0947 ($n=40$)
Apr. 1, -0.5116 ± 0.0784 ($n=40$)
Apr. 27, -0.5888 ± 0.0696 ($n=40$)
June 1, -0.6096 ± 0.0678 ($n=39$)
On all samples, -0.2264 ± 0.0452 ($n=199$)

Hence, there is a small negative correlation between the inorganic phosphorus and calcium in the blood of steers fed on the rations considered in this work. It becomes greater as the experiment progresses; however, more work is necessary before it can be said to have a biological significance.

There is a close relationship between the phosphorus intake of these animals and the inorganic phosphorus of the blood plasma, which bears out the findings of others (*11*, *19*). Analyses of blood for its inorganic phosphorus content can be used as a measure to determine the phosphorus needs of animals. This, however, requires further work to standardize the factor for optimum concentration under varying conditions. The age of the animal is known to influence the inorganic phosphorus of the blood (*7*), and the conclusion is quite well-founded that gestation is without effect (*16*, *18*). However, the effect of sex and pregnancy upon the normal inorganic phosphorus of the blood is not so well known.

At the end of the 150-day period, the ration of the cattle receiving pressed beet pulp, beet molasses, alfalfa hay, and salt (lot 1) was supplemented with 0.1 pound of steamed bone meal daily. After being on this ration for 30 days, blood samples were taken and analyzed for calcium and inorganic phosphorus. These results are given in table 4.

TABLE 4.—*Milligrams of calcium in 100 cc of blood serum and of inorganic phosphorus in 100 cc of blood plasma in the blood of steers, July 1, 1933.*

Steer no	Calcium	Phosphorus	Steer no	Calcium	Phosphorus	Steer no.	Calcium	Phosphorus
40	13.3	3.4	3	14.5	2.4	50	13.0	2.4
6	12.5	3.9	49	13.3	2.0	22	12.5	3.5
34	12.5	3.3	46	12.0	3.9	5	13.0	3.3
12	13.0	3.4						

The calcium of the blood remained quite constant. The inorganic phosphorus of the blood increased; at the close of the experiment, however, it was still far below that of the animals which had received steamed bone meal from the beginning of the experiment. Undoubtedly, due to the deficient ration, other tissues of the body, in addition to the blood, had been depleted of their phosphorus; consequently, time apparently is required to bring the tissues back to normal.

SUMMARY

Forty head of grade beef steers, averaging 585 pounds in weight and of similar breeding and previous treatment, were sorted into 5 groups as nearly alike as possible as to weight, quality, and condition and were fed for 150 days on rations of pressed beet pulp, beet molasses, alfalfa hay, and salt, to which was added for four of the groups a supplement of cottonseed cake, steamed bone meal, mill-run bran, and ground barley, respectively.

Blood samples were taken monthly and analyzed for calcium and phosphorus. Before going on these rations the averages per 100 cc of blood serum and plasma were: Calcium from 12.25 to 13.13 mg and inorganic phosphorus from 2.41 to 3.01 mg. Phosphorus supplements fed in the form of cottonseed cake, steamed bone meal, and mill-run bran increased the blood inorganic phosphorus to near that reported as optimum by some workers or 5 mg per 100 cc of blood serum. It was lowest in the lot receiving ground barley and highest where steamed bone meal was fed. The various phosphorus supplements produced little, if any, effect upon the blood calcium. A low negative

correlation was found between the inorganic phosphorus and calcium of the blood. There was a close correlation between the phosphorus intake and the inorganic phosphorus of the blood. The question is raised: Is it possible that many cattle (even in Utah where the soil in most cases is rich in phosphorus) may lack phosphorus in their ration? It appears feasible that this could be determined by blood analyses.

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SURVIVAL OF BLISTER-RUST MYCELIUM IN WESTERN WHITE PINE[1]

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INTRODUCTION

Studies on the growth and behavior of cankers of white pine blister rust (*Cronartium ribicola* Fischer) on western white pine (*Pinus monticola* Dougl.) have been reported by the senior writer in a previous paper.[3] That paper contains data on the manner in which canker growth rates are influenced by the size and vigor of the infected stem, by regional site conditions, and by girdling and subsequent "flag formation"—a term descriptive of the conspicuous red-brown color of the foliage of the affected part after death. In that paper (p. 594) reference was made to a study of the survival of the mycelium in trunk cankers; the results of that study are presented herein.

PROCEDURE

During 1922 and 1923 the senior writer observed that blister-rust cankers on branches of western white pine in British Columbia continued to grow toward the bole of the tree after the outer portion of the branches had died and turned into "flags", and in some cases after squirrels had girdled or severed the branch at the center of the canker.[4] In order to verify the results of these observations and to determine the extent to which the rust could continue to live and grow after all source of food supply had been cut off beyond the point of infection, a number of cankered branches were so cut that part of the infected bark remained on the stub and these stubs were examined at fixed intervals. On April 25, 1924, 34 infected branches were selected on thrifty western white pines at Chee Kye,[5] British Columbia, their diameters at the base of the canker were measured, and they were then lopped off just above the lower limits of the typical orange discoloration which closely follows the progress of the mycelium in the bark of western white pine. Throughout the remainder of this paper all cankers shortened in this manner are referred to either as "cut" cankers or as "truncated" cankers. On October 25, or 6 months from the time of cutting, all the cankers were in good condition and had continued to extend toward the bole. The downward growth of the mycelium, as indicated by the downward extension of the discoloration of the infected bark, was carefully measured for each canker.

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[1] Received for publication Dec. 27, 1933; issued July 1934.

[2] The writers acknowledge indebtedness to C. N. Partington, J. L. Mielke, and T. S Buchanan for assistance in carrying on the field studies upon which this article is based

[3] LACHMUND, H. G. GROWTH AND INJURIOUS EFFECTS OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA. Jour. Agr. Research 48: 475-503, illus. 1934.

[4] For several years numerous investigators have observed that squirrels and other rodents frequently eat the infected bark of white pine blister-rust cankers. Occasionally they completely sever the branch at the point of infection.

[5] Chee Kye is a stop on the Pacific Great Eastern Railway, approximately 40 miles, air line, north of Vancouver, British Columbia.

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In April 1925 a series of 50 uncankered thrifty branches on the same area were cut in the same way that the cankers were cut the preceding year. These checks were established to determine how long uninfected branches would remain alive after cutting. In July, 42 were dead and the remaining 8 died within 2 months. During this time most of the stubs of the cankered branches cut in 1924 remained alive and the mycelium continued its growth from their cut ends toward the boles. This proved beyond a doubt that it was the presence of the mycelium in the cut cankered stubs which had kept them alive.

In the latter part of September 1925 a forest fire swept over the area and terminated the entire study before the 34 cut cankers were remeasured. Therefore growth measurements were secured for a 6-month period only—from April 25 to October 25, 1924. A comparison of the growth rate of these cut cankers with that of normal uncut cankers in the same area indicated that the downward growth rate of the cut cankers was somewhat less in each diameter class than it was for the corresponding uncut cankers. The data were too few to be conclusive; therefore plans were made for a more complete study.

A western white pine infection area near Owl Creek,[6] British Columbia, was chosen for the second experiment. Pine infection first occurred there in 1917, increased considerably in 1921, and became common in 1923 and 1924. In 1927 at the beginning of the test the white pines were young and still thrifty. They were approximately 12 to 25 years old and from 9 to 22 feet tall.

On May 6, 1927, 100 cankers on thrifty branches of 32 trees were carefully selected. All these cankers were of 1923 or 1924 origin and were therefore relatively young, most of them having produced aecia only once or twice. In general, they were one internode distant from the bole; a few, however, were almost two internodes distant. The distance from the bole to the lower limits of the cankers ranged from 2 to 17.3 inches, being more than 8 inches in only a small number of cases. The diameter of the branches at the lower limits of the cankers ranged from 0.2 to 0.9 inch. For each 0.1-inch class between these limits the number of branches was approximately the same.

Each branch was severed by a cut perpendicular to its long axis at a point about one half of an inch above the lower limit of the discoloration. On the same trees, 100 uninfected branches were cut in identical fashion to serve as checks. The check for each cut canker was on a branch of the same size and corresponding location in the tree.

In no case were any living needles or small side branches left between the cut surface and the bole; therefore it was impossible for any of the cankered stubs or checks to obtain food except from the main stem, or bole.

These truncated cankers and checks were examined in the fall of 1927 and in the spring and fall of each successive year until the spring of 1932. The condition of each was noted—whether living or dead—and, in the case of the living cankers, the downward growth from the lower limit at the time of cutting or at the time of the preceding examination was accurately measured. Of the original 100 cankers,

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[6] Owl Creek is a stop on the Pacific Great Eastern Railway, approximately 35 miles north of Chee Kye.

22 were discarded before the completion of the study because of the death of the trees on which they were located.

On May 1, 1928, a similar experiment was begun on the same area but on different trees. Fifty cankered branches and fifty uninfected checks were cut in exactly the same way as were those in the preceding year. In order to determine the immediate effect of cutting, this series was examined monthly until fall; after that the data were taken as for the 1927 series. Here again the basis for comparisons was reduced through the death of some of the trees. Data were taken on 44 cut cankers and checks in 1929 and on 32 cankers and checks in 1930. At the end of 1930 work on this series was terminated.

SURVIVAL OF TRUNCATED CANKERS

The data summarizing the condition of the cankered stubs at half-yearly and yearly intervals are shown in table 1. The condition of the cankered stubs is assumed to be the same as that of the canker itself—i.e., living or dead—for *Cronartium ribicola* is an obligate parasite and can live only so long as its host remains alive. When a canker had grown down the stub and entered the bole, the stub died in a short time. Inasmuch as the purpose of this study was to determine the effect of cutting on canker survival and growth, those cankers that died or entered the bole were not examined at subsequent dates. Because of the uniformity of the data after the 2-year period, the half-yearly intervals are not shown.

TABLE 1.—*Survival of truncated cankers and checks, Owl Creek, British Columbia*

Cankers or checks (number)	Time after cutting	Cankers			Checks dead
		Living but not yet reaching bole	Dead before reaching bole	Entered bole	
	Years	*Percent*	*Percent*	*Percent*	*Percent*
122	½	95.9	0.0	4.1	95.9
122	1	91.0	1.6	7.4	99.2
122	1½	66.4	8.2	25.4	100.0
110 [a]	2	44.6	18.2	37.3	
78 [b]	3	21.8	21.8	56.4	
78	4	5.1	29.5	65.4	
78	5	3.8	30.8	65.4	

[a] Trees containing 12 cankers died during winter.
[b] Examination of 1928 series discontinued.

Examination of the data for the 122 checks shows that 95.9 percent, or 117, were completely dead half a year after they were cut. Only 1 of the remaining 5 checks survived for 1 year, and it died shortly thereafter.

Comparison of the survival data for the truncated cankers with those for the checks clearly shows that the former remained alive for a very much longer time than the latter. This comparison is further strengthened by the results of the monthly examination of the checks in the 1928 series. Five percent of them were dead 3 months after cutting and 90 percent in 4 months, showing that the great majority of deaths occurred during the first 6 months.

In a previous reference to the present study the statement was made [7] that the survival of the cankered stubs suggested that "the presence of the infection at the end of the stub had the effect of stimulating a reversal of flow of the elaborated food materials." The data presented herein are believed to justify fully this hypothesis. The fact that with one exception the uninfected checks died within a year after cutting whereas the cankered stubs remained alive in some cases for at least 5 years shows that the presence of blister-rust infection tended to keep the stubs alive until the cankers could grow into the bole.

The action of the rust upon the stream of assimilates in the cut cankered stubs would be an interesting subject for physiological study. No other phenomena even remotely analogous to the survival of the cankered stubs are known to the writers. Certain features of resemblance are suggested by the well-known action of false-mistletoe infection, which has the effect on several hosts of prolonging the life of certain branches; notably in the case of the lower branches of *Pinus ponderosa* Dougl. and *P. contorta* Dougl. But in these cases the prolongation is attributable to a stimulation of the growth of the foliage beyond the point of infection whereby the infected portions are provided with an increased supply of elaborated food from the normal direction.

DOWNWARD GROWTH OF TRUNCATED CANKERS

As previously stated, the downward growth of all living cut cankers was carefully measured at the time of each examination. These measurements, too numerous for detailed publication, indicate three things: (1) The downward growth rate of the mycelium in the cankered stubs was constant as long as the stubs remained alive; (2) this rate was very slightly less than the downward growth rate of normal uncut and unflagged cankers; and (3) it was almost identical with the downward growth rate of flagged cankers, which is likewise slightly less than the normal growth rate. This reduced rate of growth can obtain, however, only while the fungus is in the stub or in those portions of the stem below the flag which are in low vigor because of the flagging. As soon as the infection reaches the bole or the region of the healthy living side branches below the canker, its rate of growth immediately becomes normal.

While similar in rate of downward growth, the cut and the flagged cankers are not exactly comparable as regards the length of time they may remain alive on internodes unsupported by living foliage beyond the canker. The death of the portion of the branch or stem beyond the canker in flagging reacts differently and evidently saps the vitality of the lower uninfected portion much more than when the branch or stem beyond the canker is cut off. For example, when a canker is situated so far down on a branch that most of the foliage is beyond the point of infection, the entire branch is generally killed at the time of flag formation. It has been noted, further, that flagged cankers almost invariably die out if the length of unsupported internode is so long that the canker cannot grow over it within 2 years. On the other hand, the present data show that the majority of the cut cankers remain alive for at least 2 years and that many of them survive for 3 years or more. From these observations it appears that cutting causes less of a drain on the lower stem than does flagging. Consequently the longevity and survival data for the cut cankers cannot be used as criteria for the survival of cankers after flagging.

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[7] LACHMUND, H. G. See footnote 3.

SUMMARY

The mycelium of white pine blister rust continues to live and grow in infected western white pine bark after flagging, i.e., the death of the portion of the branch beyond the canker, has taken place or after squirrels have girdled or severed the branch at the center of the canker. In order to obtain definite information on this phenomenon the writers in the spring of 1927 and 1928 shortened 150 cankers, leaving stubs with about one half of an inch of living canker at their tips. Checks were cut in exactly the same way on uninfected branches in the same relative position in the trees.

The checks were practically all dead within 1 year from the date of cutting, whereas the cankered stubs remained alive for periods ranging up to 5 years, or until the mycelium entered the bole of the tree. The mycelium apparently stimulated a reversal of the flow of assimilates in the infected stubs.

Measurements of the downward growth of the cankers in the stub indicate that it proceeds at a constant rate slightly less than that of normal cankers but almost identical with that of flagged cankers. Flagged cankers survive for a shorter time than cut cankers.

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EFFECT OF LEAF RUST (PUCCINIA TRITICINA) ON YIELD, PHYSICAL CHARACTERS, AND COMPOSITION OF WINTER WHEATS[1]

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INTRODUCTION

There is little published information regarding the effect of the various cereal rusts on the composition of the host plants and grain, although several studies on the effect of rust on yield have been published. At La Fayette, Ind., the season of 1931 was extremely favorable for a study of the effect of leaf rust of wheat (*Puccinia triticina* Eriks.) on the composition of plants and grain and on the yield of grain and straw. A severe leaf-rust epiphytotic developed on susceptible varieties soon after the flowering period. Other diseases either were entirely absent or were present in an unimportant degree. The season was favorable for large yields. The plants developed a strong growth of straw that withstood severe winds without lodging, a particularly fortunate condition for studying the effect of rusts on cereals. A similarly fortunate combination of circumstances may not reoccur soon, which is the justification for presenting the data for a single year. A preliminary report of this work has been published by the writers (*4*).[2]

METHODS AND MATERIALS

In the fall of 1930, seven varieties of winter wheat, varying in relative susceptibility to leaf rust from extremely susceptible to highly resistant, were sown in 2 series with 4 replications of each variety in each series. The varieties were Fulhard (C.I.[3] no. 8259), Gladden, Kawvale (C.I. no. 8180), Nittany (Pa. no. 44), Purkoff, Shepherd (C.I. no. 6163), and Fultz selection (C.I. no. 11512), a highly leaf-rust-resistant selection from a segregating strain of Fultz. Beginning the following April, one series was dusted with sulphur in order to control leaf rust, whereas the other was not dusted.

The seed was sown by hand at the rate of 6 pecks per acre, this rate being commonly used in Indiana farm practice. Care was exercised to secure uniform distribution in the drill row. The two series were sown on a uniform area separated by three rows of wheat

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[2] Reference is made by number (italic) to Literature Cited, p. 1071.

[3] C.I. denotes accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

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to guard against the drifting of sulphur from the dusted to the nondusted series. Each replication or plot of a given variety consisted of four 18-foot rows 1 foot apart, the 2 outermost serving as guard rows between the varieties. The plots were continuous, i.e., separated by only 1 foot, as were the rows within the plots. The four replicated plots of each variety in both dusted and nondusted series were in linear arrangement and extended the entire length of the experimental area.

The grouping of the dusted and nondusted plots in separate series was to prevent the sulphur from drifting to the nondusted plots. This plan made impossible the more desirable systematic distribution of plots commonly in use in comparative yield trials. However, because of the uniformity of the soil and the small land area involved, this system apparently has given satisfactory data from a practical point of view.

A 2-12-6 fertilizer was applied with a disk drill at the rate of 200 pounds per acre prior to seeding. The drill was drawn parallel to the short dimension of the area, whereas the seed was drilled in the opposite direction, to avoid, so far as possible, the influence of any irregularity in the distribution of the fertilizer.

A sulphur dust containing the oxidizing agent, potassium permanganate, was used throughout the experiment. The dust was applied with a bellows-type hand duster at the rate of 100 pounds per acre, the rate being slightly increased in the later dustings. Because there is a rather large wastage of dust in using a bellows-type duster and in treating rows spaced 1 foot apart, heavy applications were necessary. The control of leaf rust was very satisfactory, the percentage of infection reaching only 10 to 15 percent on the dusted plots of very susceptible varieties, in contrast to 100 percent, shortly after flowering time, on the nondusted plots.

In this study the sulphur-dusted plots have been considered as controls, to be used as a basis from which to measure the effect of the rust on the rusted (nondusted) plots because the interest is primarily in the effect of the rust rather than that of the fungicide. It is recognized that the sulphur dust may have slightly altered the normal development of the control plants. The control plots were dusted 11 times during the period of development from the jointing stage to maturity. The dust was applied whenever it seemed necessary because of removal by rains.

In addition to the above-described sowing, a very leaf-rust-susceptible selection of Michigan Amber wheat was sown in the same block in 8 plots of 16 rows each. One extra row between plots and the outermost unharvested rows of each plot served to guard against sulphur drifting. By variations in the time at which the dusting was started in the several plots, four different degrees of severity of rust were produced. These plots were in duplicate for each of the dusting treatments. The plots dusted, beginning at the jointing stage, booting stage, and flowering stage, and the nondusted plots developed 15, 52, 100, and 100 percent, respectively, of leaf rust at maturity. Although no difference could be seen in the amount of rust at maturity on the plants of the last two treatments mentioned, the rust developed to its maximum later on the plants dusted at flowering time, and the foliage remained alive longer than on the nondusted plants.

Notes on leaf rust were taken for each variety for each treatment on June 16, 20, and 27 and July 1. On the first date little killing of the upper leaves had occurred as a result of ripening or rust attack. By June 27 most of the leaves were dead or entirely chlorotic. The average number of living leaves per tiller and the leaf-rust severity for each living leaf per tiller were estimated for a number of representative tillers on these different dates. Rust severity was estimated according to the scale adopted by the Division of Cereal Crops and Diseases. The percentages of rust for June 16 represent the average estimated amount of rust per tiller on leaves living at that time. The percentages of rust infection recorded for June 20 and 27 and July 1 are based on the maximum amount of rust that had developed prior to any one of the given dates on all of the leaves per tiller that were alive on June 16, even though some or all of them may have been dead when the notes were taken on the later dates.

Yield data were taken from the 2 center rows of the 4-row plots and from 12 center rows of the 16-row plots. The ends of the rows for a distance of 1 foot were discarded in harvesting the plots in order to avoid border effect. In presenting the yield data the probable errors of the means of the replicate 4-row plots have been calculated by the deviation-of-the-mean method (*6*).

Detailed methods pertaining to separate phases of the study are given later.

EFFECT OF LEAF RUST ON YIELD

GRAIN

Leaf rust was responsible for very marked decreases in yield of winter wheats, as determined by the comparison of replicated control (sulphur-dusted) plots and heavily rusted plots. The pertinent data are given in tables 1 and 2. The yields of the susceptible varieties, Shepherd, Michigan Amber, and Gladden, were reduced 28.4, 20.8, and 14.8 percent, respectively. The reduction for Nittany, moderately susceptible, was 8.2 percent and for the slightly more resistant Purkoff only 2 percent.

The control (sulphur-dusted) plots of the more resistant varieties, Kawvale and Fultz selection, yielded less than the rusted (nondusted) plots (table 1).[4] The differences amounted to 3.4 and 12 percent, respectively. The difference for Kawvale is not statistically significant, but that for Fultz selection is 3.3 times its probable error. Likewise, the yield of the rusted plots of Fulhard slightly exceeded that of the control plots, although the former were very severely rusted and dusting might have been expected to increase the yield. The lower yields of the control plots of these three varieties suggest the possibility of an inhibitory action of the sulphur on the plant development and that the effect may vary with different varieties.

The reduction in yield of Michigan Amber was closely proportional to the estimated leaf-rust severity (table 2 and fig. 1). Thus, the plots having infections of 15, 52, and 100 percent of late development and 100 percent of earlier development produced average yields of 38.4, 34.2, 31.3, and 30.4 bushels per acre, respectively.

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[4] The nondusted plots of Fultz selection, in conformity with the nondusted plots of other varieties, are designated in the tables as rusted plots, although little rust developed on them because of their natural resistance.

TABLE 1.—*Effect of leaf rust on yield per acre of grain, protein, and straw, test weight per bushel, and weight per 1,000 kernels of winter-wheat varieties of differing degrees of susceptibility to leaf rust*

Variety	C.I. no.	Kind of plot	Maximum leaf-rust severity	Acre yield of grain [a]	Grain loss or gain in rusted plots	Test weight per bushel	Weight [b] per 1,000 kernels	Loss or gain in weight per 1,000 kernels in rusted plots	Acre yield of protein	Protein loss or gain in rusted plots	Acre yield of straw [c]	Straw loss or gain in rusted plots
			Percent	*Bushels*	*Percent*	*Pounds*	*Grams*	*Percent*	*Pounds*	*Percent*	*Pounds*	*Percent*
Shepherd	6163	Rusted	100	29.2±0.71	−28.4	58.5	30.9	−7.5	156.7	−38.3	4,852±142	−13.3
		Control	13	40.8± .99		59.6	33.4		254.1		5,595±163	
Gladden		Rusted	100	37.3± .91	−14.8	57.6	31.3	−12.1	185.0	−25.6	4,811±141	−14.2
		Control	15	43.8±1.06		59.1	35.6		248.6		5,606±164	
Fulhard	8259	Rusted	100	35.1± .84	+1.2	62.2	30.9	−5.2	188.5	−14.6	4,762±139	−7.6
		Control	10	34.7± .85		62.9	32.6		220.6		5,152±150	
Nittany		Rusted	75	34.5± .84	−8.2	58.1	41.4	−3.0	194.4	−15.5	5,602±164	−10.7
		Control	10	37.6± .91		58.7	42.7		230.0		6,274±183	
Purkoff		Rusted	65	39.5± .96	−2.0	58.6	31.2	−4.9	212.5	−11.8	5,467±160	+.3
		Control	8	40.3± .98		59.2	32.8		240.8		5,452±159	
Kawvale	8180	Rusted	45	42.2± .99	+3.4	60.6	32.8	−3.2	228.1	−0.6	4,950±145	−.6
		Control	5	40.8±1.03		60.9	33.9		229.5		4,980±145	
Fultz selection	11512	Rusted	7	43.0± .93	+12.0	59.2	32.0	−1.5	245.8	+10.3	5,872±160	+7.3
		Control	1	38.4±1.05		58.8	32.4		222.8		5,472±172	

[a] Average of 4 replicated plots.

[b] Average, on air-dry basis, of 4 replicated plots of Shepherd, Nittany, and Fultz selection; determined on composite samples of threshed grain from 4 replicated plots of Gladden, Fulhard, Purkoff, and Kawvale.

[c] Average of 4 replicated plots on air-dry basis at threshing time.

TABLE 2.—*Effect of leaf rust in different degrees of severity on yield of grain and protein per acre, test weight per bushel, and weight per 1,000 kernels of Michigan Amber wheat* [a]

Stage of plant maturity at beginning of dusting	Maximum leaf-rust severity	Acre yield of grain	Grain loss in rusted plots [b]	Test weight per bushel	Weight per 1,000 kernels [c]	Loss in weight per 1,000 kernels in rusted plots [d]	Acre yield of protein [d]	Protein loss in rusted plots [b]
	Percent	*Bushels*	*Percent*	*Pounds*	*Grams*	*Percent*	*Pounds*	*Percent*
Nondusted	100	30.4	20.8	58.2	32.3	8.24	163.3	30.2
Flowering	100	31.3	18.5	58.5	32.9	6.53	173.9	25.6
Booting	52	34.2	10.9	59.3	34.4	2.27	197.8	15.4
Control (jointing)	15	38.4		59.7	35.2		233.8	

[a] Averages of determinations from duplicate 12 rod-row plots.
[b] Percentages calculated on basis of the plots in which dusting was begun at the jointing stage.
[c] Determined on air-dry basis.
[d] Determined on dry-weight basis.

STRAW

When the grain was threshed the total weights of the harvested bundles, including grain, were recorded. At that time the bundles had dried in the field for 8 days under excellent drying conditions and were apparently air-dry. The recorded yields of straw (table 1) are average weights for four replicated plots based on these bundle weights minus the weight of the grain threshed from them. The probable errors were calculated in the same manner as were those for the grain yields.

The effect of leaf rust on yield of straw of each of the several varieties in the experiment was very similar to that on the yield of grain. In the susceptible varieties, Shepherd and Gladden, the yield of straw was reduced 13.3 and 14.2 percent, respectively, and in the less susceptible Nittany, 10.7 percent. In the moderately resistant varieties, Kawvale and Purkoff, no appreciable difference was noted between the yields of straw from the rusted and the control plots. The rusted plots of Fultz selection, the highly resistant variety, yielded 7.3 percent more straw than did the control plots.

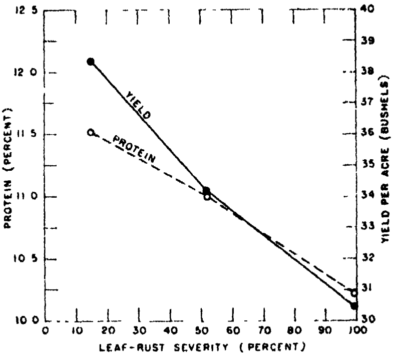

FIGURE 1.—Relation of leaf-rust severity to yield and protein content of Michigan Amber wheat.

The straw yields of the replicated plots varied considerably and thus led to a much larger probable error in relation to yield than was found for the grain yields. From a statistical standpoint the effect of rust on the straw yields is of doubtful significance. The effect, however, is in harmony with that for the yields of grain.

NATURE OF YIELD REDUCTION CAUSED BY LEAF RUST

The effect of leaf rust on the number of kernels and weight of grain per head was studied in the following three varieties of winter wheat: Shepherd (susceptible), Nittany (moderately susceptible), and Fultz selection (highly resistant). In addition, the effect of varying severities of leaf-rust infection on kernel number and weight was studied in Michigan Amber (highly susceptible). The variations in the dusting procedure to produce these varying severities of rust are described above The pertinent data for all varieties are given in table 3.

REDUCTION OF NUMBER OF KERNELS PER HEAD

The most heavily rusted plants of the highly susceptible Michigan Amber suffered an average loss of 4.65 kernels per head as compared with the control plants, or a reduction of 16.69 percent. The average numerical kernel loss per head in Shepherd was 4 91 kernels, but, owing to the larger heads, the percentage loss was 13 85 percent, or less than in Michigan Amber (table 3). These losses appear to be statistically significant. In the less susceptible Nittany the loss was only 1 13 kernels per head, or 3.91 percent The loss was statistically significant, however

In the very resistant Fultz selection the difference in number of kernels was in favor of the rusted plants, as it was in yield, but this difference was small and not significant. In Michigan Amber the percentage reduction in number of kernels per head was found to vary regularly in proportion to the rust severity.

REDUCTION IN WEIGHT OF KERNELS

Leaf rust also markedly reduced the weight of kernels in the susceptible varieties Thus, the weights of the individual kernels from rusted plants of Shepherd and Michigan Amber were reduced on an average of 5.95 and 7 10 percent, respectively, as compared with kernels from the control plants. The loss in weight per kernel of Nittany was only 1.53 percent, whereas that of the rusted plants of the resistant Fultz selection again, as in number of kernels, very slightly exceeded that of the control plants, the kernels being 0.92 percent heavier than those of the controls. Reductions in kernel weight also were proportional to rust severity, as is shown in the Michigan Amber series, although the most important effect of rust on yield was expressed in a numerical reduction of kernels per head. The reduction in weight was not caused by a visible shrinkage of the kernels, for kernels from rusted plants apparently were as plump as those from the control plants.

Mention should be made here of the comparison between weight per kernel presented in table 3, as determined from the 100-head samples of the larger heads of uniform size taken from the standing grain, and the weight per kernel as determined from 1,000 kernels of the threshed grain (tables 1 and 2). The weight of grain in grams per 1,000 kernels as given in tables 1 and 2 may be compared directly with the data in table 3. As would be expected, in every case the weight per kernel of the selected heads is greater than that of the threshed grain. On either basis, however, the same trends may be noted in the effect of rust on kernel weight.

A similar comparison may be made between the reduction in yield per acre of threshed grain (tables 1 and 2) and the reduction in weight of grain per head calculated from the samples of 100 heads (table 3). In Michigan Amber the agreement in percentage losses determined by the two methods is very close, while in Shepherd and Nittany there are considerable differences. Since the data from the threshed grain of the entire plot must be considered the more reliable, the results from the relatively small lots of 100 heads should be accepted as indicative of trends rather than as representative of the actual conditions within the plots.

TABLE 3.—*Effect of leaf rust on kernel formation in winter wheats* [a]

Variety	C. I. no.	Kind of plot	Maximum leaf rust severity	Kernels per head		Weight per kernel		Total weight of kernels [b] per head		Proportion of total yield loss caused by—	
				Mean	Loss or gain in rusted plots	Mean	Loss or gain in rusted plots	Mean	Loss or gain in rusted plots	Reduction in kernel number	Reduction in kernel size
			Percent	*Number*	*Percent*	*Mg*	*Percent*	*Mg*	*Percent*	*Percent*	*Percent*
Shepherd	6163	Rusted	100	30.53±0.31	−13.85	32.56	−5.95	994±9	−18.99	72.96	27.04
		Control	13	35.44±.33		34.62		1 227±11			
Nittany		Rusted	75	27.77±.22	−3.91	43.82	−1.53	1 217±9	−5.37	72.88	27.12
		Control	10	28.90±.24		44.50		1 286±11			
Fultz selection	11512	Rusted	7	31.42±.36	+2.45	32.97	+.92	1 036±11	+3.39	72.76	27.24
		Control	1	30.67±.26		32.67		1 002±8			
Michigan Amber		Nondusted	100	23.20±.18	−16.69	33.62	−7.10	780±6	−22.61	73.82	26.18
		Dusted in flowering stage	100	24.33±.19	−12.64	33.91	−6.30	825±7	−18.15	69.63	30.37
		Dusted in booting stage	52	25.48±.20	−8.50	35.28	−2.51	899±6	−10.81	78.71	21.29
		Control (dusted in jointing stage)	10	27.85±.22		36.19		1 008±8			

[a] Determined on composite samples of 100 uniform, ripe heads selected partly from each of 4 replicated plots of Shepherd, Nittany, and Fultz selection and from duplicate plots of Michigan Amber.

[b] Determined on air-dry basis.

RELATIVE IMPORTANCE OF REDUCTION IN WEIGHT AND NUMBER OF KERNELS

The loss in yield of grain per head caused by leaf rust through reduction in number of kernels has been calculated by multiplying the mean weight per kernel of the grain from the control plants by the mean loss of kernels per head of the rusted plants. The loss through reduction in size of kernel has been calculated by multiplying the mean loss in weight per kernel of the rusted plants by the mean number of kernels per head of the rusted plants. The sum of these losses (table 3) equals the mean total loss per head.

In all cases where leaf rust caused a loss, the reduction in number of kernels accounted for approximately three-fourths of the loss per head and the loss in kernel weight the remainder (table 3). This approximate ratio changed but little, regardless of the severity of the infection or the susceptibility of the varieties. Thus, in Shepherd and Nittany, which varied greatly in the severity of rust, the ratios between the two loss factors were nearly identical. In Michigan Amber the ratios varied somewhat in the plots under different rust attacks, but no significant trends in relation to rust severity are evident.

Since leaf rust was very light during the seedling stage of the grain, it appears unlikely that the number of tillers or heads per plant could have been appreciably affected. This factor has, therefore, been disregarded in considering the manner in which leaf rust reduced grain yields for this year.

The findings for the susceptible varieties were very similar to those of Mains (*14*) and Johnston (*10*), who showed that leaf rust of wheat reduced the yield of grain of infected plants primarily by reducing the number of kernels per head and to a lesser degree by reducing the weight of the kernels.

EFFECT OF LEAF RUST ON PHYSICAL CHARACTERS OF GRAIN

The most marked visible effect of rust on the grain was a reduction in the proportion of vitreous kernels in the grain from rusted plants as compared with that from the control plants. This physical condition was correlated with a reduced protein percentage content of the grain as shown in tables 4 and 5. All the susceptible varieties studied were thus affected, and in all varieties the difference in kernel color made it possible to distinguish easily the grain of the rusted plants from that of the controls. In the soft wheats the kernels from rusted plants were soft and starchy throughout, whereas those from the control plants tended to have vitreous tips. In the hard and vitreous Fulhard and the semihard Kawvale a physical separation of the kernels could be made into the three classes, (1) soft or starchy, (2) piebald—those comprising mixed starchy and vitreous areas—and (3) vitreous (table 4 and fig. 2). In Fulhard 73.9 percent by weight of the kernels were vitreous and 26.1 percent were piebald for the control plants. Under severe rust attack the ratios were nearly reversed, only 20.4 percent of the kernels being vitreous and 79.6 percent piebald. The same tendency was expressed in Kawvale. This induced starchiness or piebaldness in all varieties is identical with the condition classified as yellow berry in the hard-wheat grain trade. Mains (*14*) has reported that leaf rust tends to increase the proportion of yellow-berry kernels of plants artificially inoculated with leaf rust in the greenhouse.

In Fultz selection, the resistant variety, where only 7 percent of rust occurred on the nondusted (rusted) plots, there were no visible differences between the physical characters of the grain from the control and the nondusted plants (table 5). This was correlated with distinctly similar protein and starch contents of the grain from the dusted and nondusted plants.

FIGURE 2.—*A*, Wheat from plots of rusted Fulhard plants. High percentage of yellow-berry kernels, a condition here caused directly by leaf rust. *B*, Wheat from control (sulphur-dusted) plots grown under approximately identical conditions except for the absence of leaf rust.

TABLE 4.—*Effect of leaf rust on relative percentages by weight of vitreous, piebald, and starchy kernels in hard (Fulhard) and semihard (Kawvale) winter-wheat varieties* [a]

Variety	C. I. no.	Kind of plot	Maximum leaf-rust severity	Kernel type		
				Vitreous	Piebald	Starchy
			Percent	*Percent*	*Percent*	*Percent*
Fulhard	8259	Rusted	100	20.4	79.6	
		Control	10	73.9	26.1	
Kawvale	8180	Rusted	45	10.6	60.8	29.
		Control	5	20.7	75.4	3.

[a] Determined on 20-gram samples of grain.

Heavy leaf-rust infection had only a small, although consistent, effect on the test weight per bushel of the grain of the susceptible varieties. Gladden and Michigan Amber were most affected, both being reduced in test weight by 1.5 pounds per bushel. In test weight, as in yield, Fulhard was unaffected by the heavy rust in spite of the fact that the rust caused the production of a high proportion of yellow-berry kernels. The test weight of Fultz selection was slightly higher for the rusted plot.

TABLE 5.—*Effect of leaf rust on chemical composition of mature grain of winter-wheat varieties of differing degrees of susceptibility to leaf rust* [a]

Variety	C.I. no	Kind of plot	Maximum leaf-rust severity	Dry weight of grain per head [b]	Protein ($N \times 5.7$)	Sucrose	Starch	Phosphorus (P_2O_5)	Total ash
			Percent	*Grams*	*Percent*	*Pct.*	*Pct.*	*Percent*	*Pct.*
Shepherd	6163	Rusted	100	0.868	10.25±0.08	1.52	71.7	1.18	2.09
		Control	13	1.070	11.90± .10	1.73	70.0	1.21	2.17
Michigan Amber		Rusted	100	.685	10.20	1.12	71.4	1.14	2.07
		Control	15	.886	11.53	1.49	70.5	1.13	2.00
Gladden	--	Rusted	100	--	9.45	1.39	72.7	1.12	1.96
		Control	15	--	10.81	1.58	69.6	1.13	1.95
Fulhard	8259	Rusted	100	--	10.15	1.37	71.7	1.13	1.95
		Control	10	--	12.00	1.52	70.5	1.17	2.10
Nittany	--	Rusted	75	1.068	10.70± .09	1.55	69.7	1.24	2.13
		Control	10	1.128	11.62± .10	1.65	67.9	1.23	2.13
Purkoff	--	Rusted	65	--	10.27	1.32	71.3	1.09	1.95
		Control	8	--	11.38	1.55	70.8	1.11	1.98
Kawvale	8180	Rusted	45	--	10.19	1.29	72.2	1.12	1.87
		Control	5	--	10.58	1.43	70.6	1.09	1.88
Fultz selection	11512	Rusted	7	.913	10.90± .09	1.21	68.7	1.14	1.94
		Control	1	.876	11.06± .09	1.25	69.0	1.16	2.01

[a] Determined on the threshed grain from each of 4 replicated plots of Shepherd, Nittany, and Fultz selection, on a composite sample of threshed grain from 4 replicated plots of Gladden, Fulhard, Purkoff, and Kawvale and on threshed grain from duplicate plots of Michigan Amber.

[b] Determined on the oven-dry basis on a composite sample of 100 ripe heads from 4 replicated plots of Shepherd, Nittany, and Fultz selection and from duplicate plots of Michigan Amber

EFFECT OF LEAF RUST ON CHEMICAL COMPOSITION OF GRAIN AND COMBINED CULMS AND LEAVES

METHODS

A chemical study was made on the kernels and combined culms and leaves of three varieties, including the susceptible Shepherd, the moderately susceptible Nittany, and the highly resistant Fultz selection, under conditions of severe and light leaf-rust infection. A similar study of the effects of different degrees of leaf-rust severity was made on the susceptible variety, Michigan Amber. In these studies the plants were sampled for analyses when in the early milk, late milk, and dough stages. In addition, analyses were made of the threshed grain of the above-mentioned varieties together with that of Gladden, Fulhard, Purkoff, and Kawvale, also grown under conditions of severe and light leaf-rust infection. The seeding methods and the rust-control methods are described above.

Samples for analysis consisted of 100 tillers selected from the replicated plots, except on June 16, when they consisted of culms and leaves of only 50 tillers and heads of 100 tillers. Through error only 75 heads of Nittany were sampled on that date. Only the larger tillers that appeared to be in a uniform stage of development were selected. The culms were clipped as close to the ground as possible. A proportionate part of the 100 tillers of each sample was taken throughout the central 16 feet of one row in each of the replicated plots. In order to avoid the exceptionally rank plants at the row ends, no tillers were taken from either end of the rows for a distance of 1 foot. Outside rows only, except for Michigan Amber, were sampled, the inner two rows being reserved for determination of final yield and composition of the mature grain. Thus, each of the rows sampled in the early milk, late milk, and dough stages was bordered on one side by a row of the same variety and on the opposite side by that of another. This arrangement was similar in both the control and rusted plots. In the Michigan Amber series inner rows of the plots were available for taking the samples for chemical analyses.

The head samples clipped from the culms in the laboratory immediately after collections were made were dried in a vacuum oven at 70° C. When dry, the kernels were separated from the chaff by hand. The culms and leaves were weighed and finely chopped immediately after gathering. Representative samples were then taken for analysis and dropped into boiling alcohol containing calcium carbonate. At the fully ripe stage only the threshed grain was analyzed.

The samples of combined culms and leaves were analyzed for content of nitrogen, reducing sugars, sucrose, and starch. The same determinations were made on the kernels with the addition of determinations of phosphorus and total ash. Nitrogen was determined by the Kjeldahl method, phosphorus by a modification of the Neumann-Pemberton volumetric method, reducing sugars by a combination of the methods of Quisumbing and Thomas and of Bertrand, sucrose by the invertase method, and starch by the takadiastase method. The total nitrogen of the grain has been reported as protein (N × 5.7). Qualitative tests indicated the absence of nitrate nitrogen in all the materials analyzed. It is realized that the small percentages of material of the culms and leaves, determined as starch by the takadiastase method, may represent levulosans rather than true starch. Since the results of sugar and starch determinations on the immature kernels were very irregular and unsatisfactory, probably owing to enzymatic action occurring during the drying process of the heads, they are not reported here. All analytical data reported in this paper have been calculated on the dry-weight basis.

The probable errors for protein content were determined by the deviation-of-the-mean method (*5*) on the four replicated plots of Shepherd, Nittany, and Fultz selection in both the control and rusted series.

GRAIN

PROTEIN

The outstanding finding of the chemical study is that leaf rust, on susceptible or partly susceptible wheat varieties, markedly reduced the protein content of the grain. This held equally in the soft red winter wheats, as represented by the varieties Shepherd, Michigan Amber, and Gladden, in the hard red winter variety, Fulhard, and in the semihard varieties, Purkoff and Kawvale, as shown in tables 5, 6, and 7, and figures 1, 3, and 4.

This reduction in protein appeared to be in approximate proportion to the severity of the rust on the plants. It was demonstrated with Michigan Amber, in which the different degrees of severity of rust at maturity were produced by beginning the dusting treatment of different plots at successively later dates, i.e., at the jointing, booting, and flowering stages, while others were not dusted. The plots that became infected with leaf rust to the extent of 15, 52, and 100 percent of relatively late development and 100 percent of earlier development produced grain containing, respectively, 11.53, 11.00, 10.53, and 10.20 percent of protein (table 7 and fig. 1). That the percentage of protein is affected in approximate proportion to the rust severity also is shown by the other varieties, the protein content of the more susceptible varieties being very distinctly reduced, while that of the resistant varieties was not appreciably affected. The effect on the moderately susceptible and moderately resistant varieties was intermediate between these two extremes. Thus, the protein content of

Shepherd (rusted 100 percent), Nittany (rusted 75 percent), and Fultz selection (rusted only 7 percent) was 1.65, 0.92, and 0.16 percent less, respectively, than that of the controls (table 5).

The differences in protein content of the grain from the rusted and control plants became pronounced sometime between the late milk and dough stages (tables 6 and 7 and fig. 3). It was immediately before this period that the rust infection reached its maximum on most of the varieties. Small differences in percentages of protein due to leaf rust were consistently found in the grain of susceptible varieties in the late milk stage and became progressively greater as the grain approached maturity. As shown in figure 3, the protein percentage of the grain of all varieties sampled in the earlier stages fell greatly between the early milk and late milk stages for both the rusted and control plants. Thereafter the proportion as well as the total quantity (fig. 4) of protein per head rose in both the rusted and control plants. This is similar to the findings of Brenchley and Hall (*2*), Thatcher (*21*), Woodman and Engledow (*22*), Kiesselbach (*12*), and Saunders (*18*), who studied chemically the development of the normal kernel. It is to be noted, however, that the drop in the percentage of protein is not so great in grain from control plants as in grain from rusted plants, and that the subsequent rise is greater in the former.

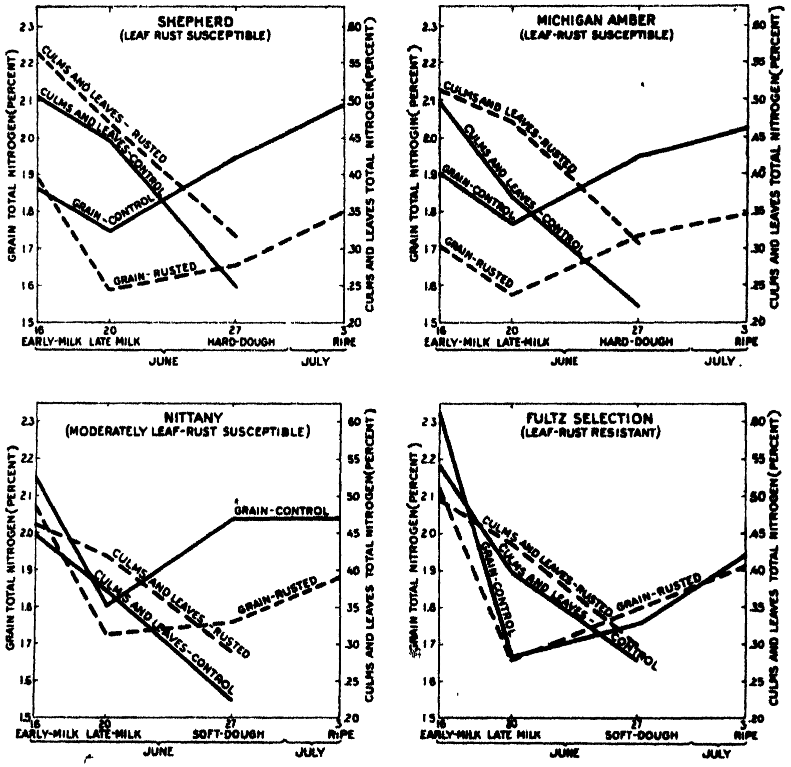

FIGURE 3.—Percentage of total nitrogen present in grain and vegetative portions of rusted and control wheat plants from the early milk stage to maturity.

This reduction in the proportion of protein was associated with a similar reduction in the total dry weight of the grain per head and in acre yield for all susceptible varieties except Fulhard, where no significant change in yield was induced by the rust (fig. 2). The accumulation of nitrogenous materials in the kernels was continuous from the early milk stage until maturity in both the rusted and control plants, but it was more rapid and greater in the controls (fig. 4).

The total acre production of protein is of interest, especially in the hard wheats, in which the relative value of the crop is in a large degree

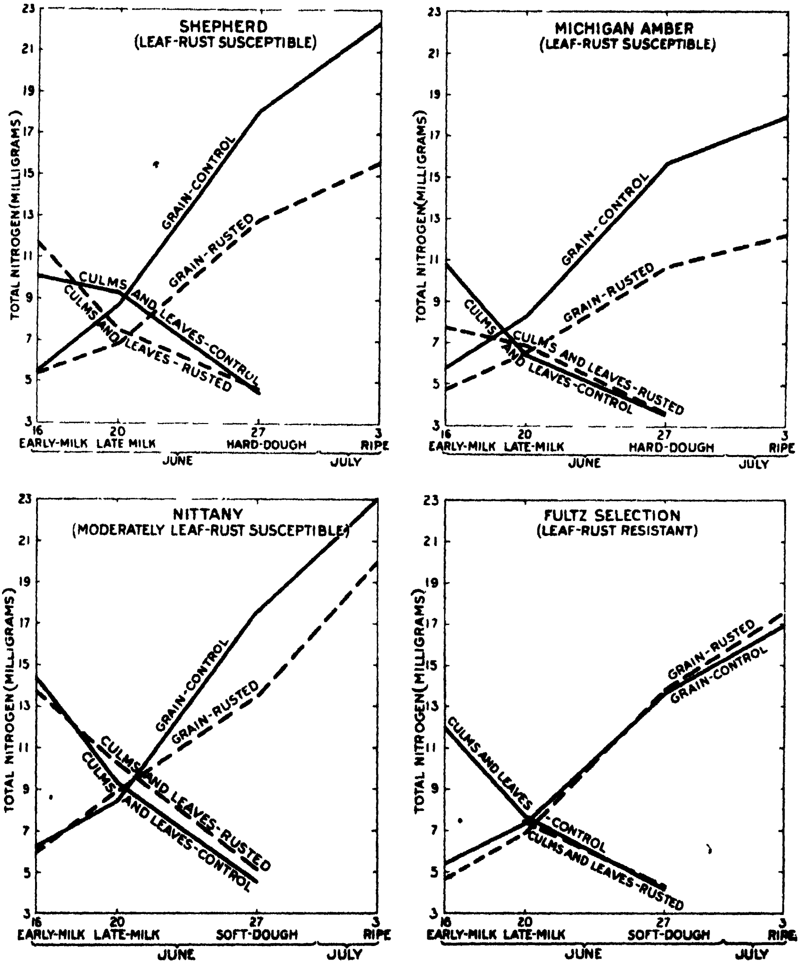

FIGURE 4.—Total quantities per tiller of total nitrogen (in milligrams) in grain and vegetative portions of rusted and control wheat plants, from early milk stage to maturity. (Since the dry weight of the samples from the rusted plot of Michigan Amber was accidentally not determined on June 1, the value given on that date is for the plots first dusted in the flowering stage, which were also severely rusted.)

dependent on the protein content. Since both the quantity of grain and the proportion of protein were reduced by leaf rust in most of the susceptible varieties, the acre yield of protein, which is a function of the two, was greatly affected. In Shepherd and Michigan Amber the yields of protein were reduced 38.3 and 30.2 percent, respectively (tables 1 and 2). In Fulhard, in which yield of grain was not reduced by rust, the protein yield was reduced 14.6 percent.

TABLE 6.—*Effect of leaf-rust on chemical composition at different stages of maturity of the kernels and vegetative parts of winter-wheat varieties of differing degrees of susceptibility to leaf rust*

Variety and C. I. no.	Date of sampling	Stage of maturity of kernels	Kind of plot	Leaf-rust severity	Living leaves per tiller	Grain[a]				Culms and leaves[b]					
						Dry weight per head	Protein (N×5.7)	Phosphorus P_2O_5	Total ash	Average dry weight per tiller	Total nitrogen	Reducing sugars	Sucrose	Total sugars	Starch
				Percent	*Number*	*Grams*	*Percent*	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Shepherd, 6163	June 16	Early milk	Rusted	95	2	0.287	10.77	1.09	2.41	2.078	0.563	3.09	13.39	16.48	1.38
			Control	10	2½	.292	10.61	1.02	2.34	1.993	.504	2.84	16.86	19.70	1.44
	June 20	Late milk	Rusted	100	1½	.431	9.06	1.01	2.18	1.599	.470	4.09	9.52	13.60	1.41
			Control	13	2½	.513	9.96	1.05	2.20	2.081	.446	3.53	13.58	17.11	1.46
	June 27	Hard dough	Rusted	100	0	.777	9.44	1.05	1.99	1.468	.316	3.41	1.54	4.95	1.13
			Control	13	½	.930	11.09	.99	1.98	1.786	.248	6.04	4.63	10.67	1.23
	July 3	Mature	Rusted	100	0	.868	10.25±0.08	1.18	2.09	....	....	....	....	....	....
			Control	13	0	1.070	11.90±0.10	1.21	2.17	....	....	....	....	....	....
Nittany	June 16	Early milk	Rusted	50	2	.288	11.79	1.15	2.66	2.972	.462	3.56	17.68	21.25	1.32
			Control	3	2½	.289	12.25	1.13	2.67	3.205	.447	3.49	19.49	22.98	1.35
	June 20	Late milk	Rusted	75	1	.513	9.86	1.11	2.32	2.458	.419	3.97	13.98	17.95	1.40
			Control	10	2	.465	10.29	1.11	2.43	2.494	.373	4.46	17.84	22.29	1.65
	June 27	Soft dough	Rusted	75	0	.765	10.05	1.12	2.13	1.778	.290	5.92	3.76	9.68	.94
			Control	10	½	.861	11.62	1.10	2.10	2.072	.223	7.45	8.49	15.94	1.09
	July 3	Mature	Rusted	75	0	1.068	10.70±0.09	1.24	2.13	....	....	....	....	....	....
			Control	10	0	1.128	11.62±0.10	1.23	2.13	....	....	....	....	....	....
Fultz selection, 11512	June 16	Early milk	Rusted	4	2½	.220	12.09	1.18	2.31	....	.495	2.44	14.54	16.98	1.43
			Control	1	2½	.232	13.25	1.06	2.33	2.221	.540	2.34	14.79	17.12	1.38
	June 20	Late milk	Rusted	7	1½	.414	9.46	1.06	2.24	1.690	.437	3.37	11.52	14.89	1.31
			Control	1	2½	.440	9.48	1.02	2.17	1.919	.398	3.31	13.40	16.71	1.54
	June 27	Soft dough	Rusted	7	0	.768	10.23	1.01	1.93	1.424	.300	3.58	3.26	6.84	1.21
			Control	1	1	.778	9.98	1.02	1.98	1.493	.277	4.35	4.73	9.08	1.20
	July 3	Mature	Rusted	7	0	.913	10.90±0.09	1.14	1.94	....	....	....	....	....	....
			Control	1	0	.876	11.06±0.09	1.16	2.01	....	....	....	....	....	....

[a] Determined in the premature stages on a composite sample of kernels of 100 heads collected partly from each of 4 replicated plots of each variety, except for Nittany on June 16, when 75 heads were analyzed, and in the mature stage on separate samples of the threshed grain from each of 4 replicated plots of each variety.

[b] Determined on a composite sample of 100 tillers (culms and leaves combined) collected partly from each of 4 replicated plots of each variety, except for June 16, when 50 tiller samples were used.

TABLE 7.—*Effect of leaf rust in different degrees of severity on chemical composition of kernels and vegetative parts of Michigan Amber wheat at different stages of maturity*

Stage of plant maturity at beginning of dusting	Stage of maturity of kernels	Date of sampling	Leaf-rust severity[a]	Estimated average living leaves per tiller	Grain[b]				Culms and leaves[c]						
					Dry weight per head	Protein (N×5.7)	Phosphorus (P_2O_5)	Total ash	Dry weight per tiller	Total nitrogen	Reducing sugars	Sucrose	Total sugars	Starch
			Percent	Number	Grams	Percent	Percent	Percent	Grams	Percent	Percent	Percent	Percent	Percent
Nondusted	Early milk	June 16	100	1	0.279	9.70	1.06	2.25		0.511	2.55	13.04	15.59	1.25
Flowering	do	do	100	1	.255	9.58	1.08	2.33	1.559	.497	2.60	12.69	15.29	1.49
Booting	do	do	52	1½	.309	9.86	1.01	2.22	2.052	.484	2.56	15.81	18.37	1.51
Control (jointing)	do	do	13	2½	.303	10.81	1.02	2.36	2.164	.495	2.58	17.54	20.13	1.38
Nondusted	Late milk	June 20	100	0	.413	8.96	1.02	2.17	1.460	.469	3.88	8.04	11.92	1.23
Flowering	do	do	100	1	.420	9.00	1.02	2.22	1.451	.438	3.79	9.41	13.20	1.40
Booting	do	do	52	1	.446	9.50	1.00	2.10	1.607	.349	3.63	12.35	15.99	1.54
Control (jointing)	do	do	15	1½	.459	10.05	1.00	2.12	1.733	.368	3.41	14.89	18.29	1.38
Nondusted	Hard dough	June 27	100	0	.614	9.88	1.10	2.07	1.195	.305	2.55	.84	3.39	1.19
Flowering	do	do	100	0	.631	10.01	1.06	1.99	1.129	.287	2.94	1.21	4.15	1.17
Booting	do	do	52	0	.703	10.55	1.04	1.99	1.167	.269	4.28	3.10	7.38	.96
Control (jointing)	do	do	15	1	.808	11.09	1.01	1.99	1.616	.220	6.17	4.50	10.68	1.10
Nondusted	Mature	July 3	100	0	.685	10.20	1.14	2.07						
Flowering	do	do	100	0	.725	10.53	1.15	2.11						
Booting	do	do	52	0	.788	11.00	1.15	2.08						
Control (jointing)	do	do	15	0	.886	11.53	1.13	2.00						

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[a] On June 16, 100 percent of leaf rust was present on both the nondusted plots and those dusted beginning at the flowering stage the epiphytotic developed earlier and therefore more severely on the nondusted plots

[b] Determined in the premature stages on a composite sample of kernels of 100 heads collected partly from each of duplicate plots and in the mature stage on separate samples of the threshed grain from duplicate plots

[c] Determined on composite samples of 100 tillers (leaves and culms combined) collected partly from each of duplicate plots except for June 15, when 50 tiller samples were used

STARCH AND SUCROSE

The greater portion of the dry weight of the mature grain consisted of starch, which varied from approximately 69 to 73 percent in the various samples analyzed. Since protein and starch are two of the major constituents of the grain, one might expect to find, as was found, that a certain fluctuation in one would be reflected in a reverse fluctuation in the other. In the susceptible and moderately susceptible varieties, from approximately 1 to 3 percent more starch was consistently found in the less proteinaceous grain of the rusted plants than in that from the control plants (table 5). On the other hand, very little difference was found between the starch content of the grain from rusted and control plants of the resistant Fultz selection. Here again there is evidence that the sulphur dusting did not affect the composition when leaf rust was not a factor.

The percentage of sucrose in the grain, in contrast to that of the starch, was consistently lower for the rusted than for the control plants, although but small percentages were found in either case In general, the most susceptible varieties showed the greatest differences In Michigan Amber the sucrose content of the mature grain was 1 49, 1.45, 1 33, and 1.12 percent for plants ranging from light to very severe in leaf-rust infection.

PHOSPHORUS AND TOTAL ASH

Leaf rust produced no significant changes in the phosphorus content of any variety in either the immature or mature grain. The same can be said of the total ash content, namely, that, although the varieties differed, the grain from the rusted plants of a given variety was very similar to that of the control plants of that variety. Since the head and acre yields of the heavily rusted plants were considerably reduced, the total quantities of ash and phosphorus per head were, of course, greater in the control plants.

COMBINED CULMS AND LEAVES

TOTAL NITROGEN

In contrast to the grain, the vegetative portions, i.e., the culms and leaves, of the rusted plants contained a distinctly higher proportion of nitrogenous components than did corresponding parts of the control plants. Thus, the rusted plants, although higher than the control plants in respect to the proportion, and usually the total quantity, of nitrogenous materials, produced a distinctly less nitrogenous grain than did the control plants (tables 5, 6, and 7 and figs. 3 and 4).

This difference in nitrogen content of the culms and leaves, caused by leaf rust, was evident in the susceptible varieties in the early milk stage and had become more pronounced at the latest period of sampling, when the kernels were in the dough stage. The greatest difference at the dough stage was observed in Michigan Amber, in which the most heavily rusted plants contained 0.305 percent of total nitrogen in the culms and leaves, as contrasted with 0.22 percent in the control plants, an actual retention of a 38.6 percent greater concentration of nitrogen in the rusted than in the control plants. In Fultz selection, the most resistant variety, the concentration of nitrogen in the rusted plants exceeded that of the control plants by only 8.3 percent. This indicates that the rust was primarily responsible for the much greater differences between the rusted and control plants of the susceptible varieties.

At the hard-dough stage the plants of Michigan Amber bearing different severities of leaf-rust infection presented a graded series as regards percentage of total nitrogen content of the culms and leaves (table 7). With slight deviations this also held in the earlier stages.

The total quantity of nitrogen retained in the culms and leaves of the rusted tillers was usually greater than that in the control tillers, even though these parts of the rusted tillers actually contained less dry matter (fig. 4 and tables 6 and 7). In contrast, the grain from these rusted tillers contained much lower quantities of total nitrogen than did that from control tillers.

In the analysis of the culms and leaves the rust mycelium and spores that still adhered to the plants were necessarily analyzed with the host-plant tissues. Since this nitrogenous material was taken from the host plant by the fungus, it seems reasonable to consider it with that of the rusted host-plant tissues. From these studies there is no means of knowing whether the excess of nitrogen in the vegetative parts of the rusted plant as compared with the control plants was largely incorporated in the fungus or held in the host-plant tissues through a possible interference with translocation or metabolism.

Sucrose

As judged from the nearly rust-free control plants and the resistant varieties (tables 6 and 7) the sucrose content of the culms and leaves normally dropped from a range of about 15 to 20 percent at the early milk stage to from one-fourth to one-half of this content at the dough stage. The rusted plants of the several susceptible varieties showed a much more decided drop in this constituent (tables 6 and 7). For example, the sucrose content of leaves and culms of the Michigan Amber control plants fell from 17.54 percent in the early milk stage on June 16 to 4.50 percent in the hard-dough stage on June 27. During the same period the rusted plants showed a drop from 13.04 to 0.84 percent in sucrose. This reduction of sucrose in Michigan Amber varied directly with the abundance of rust, as shown in table 7, which also shows that plants ranging in rust infection from light to heavy had a sucrose content of 4.50, 3.10, 1.21, and 0.84 percent at the hard-dough stage. The differences between the sucrose content of lightly and heavily rusted plants had already become evident at the early milk stage on June 16. As a check on the sulphur-dusting method of studying the effect of leaf rust on the host, the extremely resistant Fultz selection shows only a slight effect of the rust, or possibly that of the sulphur, on the sucrose content of the vegetative parts. Since the nondusted (rusted) plot of this variety had 7 percent of rust, the small difference of 1.47 percent between the rusted and control plants may be largely accounted for as an effect of the rust attack rather than as a direct effect of the sulphur on the plant metabolism.

Reducing Sugars

In contrast to the sucrose and as judged by the control plants and the resistant Fultz selection, reducing sugars in the culms and leaves normally tended to accumulate as the plants matured. This increase was approximately 100 percent from the early milk to the dough stages (tables 6 and 7). Unfortunately, no analyses were made of the vegetative parts when fully ripe. Heavily rusted plants failed to show this accumulation of reducing sugars, the percentage content remaining about the same from the early milk to the dough stages. The graded series of rust severity on Michigan Amber (table 7) produced a distinct gradient in the reducing-sugar content of the culms and leaves, with percentages of 6.17, 4.28, 2.94, and 2.55 resulting on June 27 from leaf-rust infections, ranging from light to very heavy.

STARCH

Starch, or the materials determined as starch by the described method of determination, is a relatively unimportant constituent of the culms and leaves of the wheat varieties studied, composing only about 1 to 1.5 percent of the total dry weight. Leaves and culms of the control plants, as they matured, showed no definite changes in this constituent. Leaf rust apparently has little or no important effect on these materials. It is recognized that the materials here described probably represent levulosans instead of starch.

DISCUSSION

The findings reported in this paper add further evidence to those supplied by Kightlinger and Whetzel (*13*), Mains (*14*), and Johnston (*10*) that leaf rust is a very important factor affecting yield of winter wheats in the United States. The losses of more than 28 percent in the yield of Shepherd and 20 percent in that of Michigan Amber are probably fairly representative of the destructiveness of a severe leaf-rust epiphytotic on susceptible winter wheats in Indiana. Such epiphytotics are not infrequent there, and most of the commercial varieties are extremely susceptible to this disease.

Fulhard probably was the most severely rusted variety in the test, yet its yield was not reduced. This is the more puzzling because the physical characters and chemical composition of the grain of Fulhard were more greatly affected by the rust infection than those of any other variety in the trial. Salmon and Laude (*17*) reported a similar result with Fulhard in 1929. They found a distinct relationship between leaf-rust resistance and yielding ability in 24 varieties tested for yield. Although Fulhard was one of the varieties most severely attacked by leaf rust, it produced the highest yield of the entire group.

No satisfactory explanation can be given to account for the apparent depression of yield in the sulphur-dusted plots of the Fultz selection, the highly leaf-rust-resistant variety. All of the replicated plots of this variety that were not dusted were located beside a 2-foot nursery path but protected from border effect by one guard row of the same variety. It is possible that border effect extended into the harvested rows of this variety, thus increasing the yield. However, a similar but less pronounced depression of yield in the sulphur-dusted plots occurred also in the semiresistant variety, Kawvale, in which there was no possible border effect. It is also possible that, since leaf rust was not a factor in the yield of the rusted plots, a possible injurious effect of the sulphur may have become apparent in the yield of the control plots.

Very little study has been made of the effect of cereal rusts on the composition of the infected host plants and the grain produced by them. The opinion is commonly held that both stem rust and leaf rust tend to increase the protein percentage in the wheat grain. But few experimental data have been found in the literature to support this view, and these cannot be accepted without question. In 1889 Harper (*5*), in an analysis of shrunken, "rusted or frosted", wheat from Minnesota, found that it contained more than 2 percent more protein than did the well-filled grain. Shutt (*19*), in Manitoba, and Snyder (*20*), in Minnesota, analyzed both the straw and grain of severely stem-rusted and stem-rust-free wheat plants. Both found that the percentage of crude protein was much higher in the straw and grain of rusted plants than in the straw and grain of nonrusted plants. Shutt reported that the grain from rusted wheat contained 13.69 percent of protein, as compared to only 10.50 percent in the rust-free wheat. Snyder found that the grain from heavily rusted plants contained 16.37 percent of protein as compared to 13.34 percent in the rust-free plants. Shutt found three times as much crude protein in rusted straw as in rust-free straw. Snyder's findings relative to the protein content of the straw accorded with those of Shutt. Ince (*9*), in North Dakota, found rusted straw much higher in protein than was nonrusted straw.

If stem rust tends to increase the protein content of the grain, as these data seem to show, the effect is directly contrary to that of leaf rust, which undoubtedly decreases the protein content. Neither Shutt nor Snyder states just how rusted and nonrusted plants were obtained from the one field sampled in each case. It would seem likely that under conditions of a severe stem-rust epiphytotic it would be necessary to take rusted and nonrusted samples from relatively low and high elevations, respectively, of the single field, which, if done, might alone account for the variations in protein content of the grain, as shown by Newton and Malloch (*16*).

Contrary to the findings of both Shutt and Snyder, Headden (*8*), working in Colorado, concluded that a heavy stem-rust epiphytotic in 1915 was responsible for the low protein content of certain wheat varieties grown that year as compared with the protein content of the same varieties grown in 1913, when rust was not an important factor. His conclusions were based on the fact that the two crops were grown on the same piece of land and in both years had received identical irrigation and fertilization. He found that a greater percentage of the total nitrogen of the plant remained in the straw in the rust year of 1915 than was the case in 1913. This was in general accord with the results of Shutt and Snyder. Headden attributes this condition to the interference of the rust in the translocation of nitrogen into the head. Thus, his interpretation of the effect of stem rust on the movement of nitrogenous compounds in the plant is similar to the writers' findings for leaf rust. However, it is possible that factors other than stem rust, not under control in Headden's experiments, may have been responsible for the low-protein grain of 1915. Mangels and Sanderson (*15*) concluded, on an apparently more secure basis, that stem rust tends to reduce the protein content of the grain. In 1922, when stem rust was severe at Fargo, N.Dak., they found that the rust-susceptible varieties produced grain "quite low" in protein content and that the grain of the most susceptible varieties was lowest in protein. Since the varieties studied had not shown these differences in protein percentages in years when stem rust was not a factor, they believed that stem rust was responsible for them.

Leaf rust is mentioned neither by Headden nor by Mangels and Sanderson. If present it might have been largely responsible for the low protein content attributed to stem rust. The literature reviewed on the subject apparently affords no sound basis for an opinion regarding the true effect of stem rust on the protein content of the wheat kernel.

In 1927, Broadfoot (*3*), working in Minnesota on spring wheat grown in leaf-rusted plots and plots in which the rust was partly controlled by sulphur dusting, concluded that leaf rust was responsible for a slight increase in protein content and a slight shriveling of the grain. This is contrary to the conclusion of the writers. Because of the irregularity in the protein content of the grain in the different plots of each of Broadfoot's series of dusted and nondusted plots, the small average difference (0.36 percent) in protein, which was found between the two series, appears insignificant. The shriveling of the kernels that he reports is suggestive of injury by heat and drought or by stem rust rather than by leaf rust, as shown by Mains (*14*) and Johnston (*10*) and by the writers in the present paper. In the writers' investigations at La Fayette, Ind., they found that yield losses of more than 25 percent caused by leaf rust have not been attended by any evident shriveling of the kernels.

The ultimate result of severe leaf-rust infection was the retention of a greater proportion, as well as a greater quantity, of the total nitrogenous materials of the plant within the leaves and stems of the rusted plants than within those of the control plants. The writers were unable to determine whether the retention of these materials in the vegetative parts is to be accounted for primarily as a direct result of their utilization by the fungus, as a result of direct interference of the fungus in metabolism or translocation of the nitrogenous materials, or as a combined result of these factors, because the nitrogen in the rust spores and in the mycelium cannot be determined separately from that within the host tissues. The excess quantity of nitrogen per tiller present at any one time in the rusted culms and leaves, as compared with that in the control plants, does not equal the deficiency of total nitrogen per head in the mature grain from rusted plants. This fact may be partly accounted for by the continuous loss of nitrogenous material from the rusted plants in the form of urediospores that are scattered by the wind or that often fall to the ground in quantities sufficient to give the soil a distinctly brick-red cast. It is also possible that nitrogenous materials may be more readily leached from the early-killed leaves of rusted plants than from the longer-lived foliage of control plants.

The reduced nitrogen percentage in the kernels of the rusted plants was invariably associated with an increased percentage of starch. However, the fungus reduces the total quantity of nonnitrogenous material available for storage as starch in the kernels, as is definitely shown in the total quantity of starch per head laid down in the rusted and nonrusted plants. The increased percentage of starch in the kernels of the rusted plants must be due to a differential effect of the fungus on the supply of these two classes of elaborated plant food.

Evidently, the impaired photosynthetic activity of the rusted plant, due to early death of the leaves, is not proportionately so great as is the effect of the fungus in limiting the nitrogenous materials available for storage as protein in the grain. The production of increased numbers of yellow-berry and starchy kernels by rusted plants as compared with control plants is definitely correlated with a decreased proportion of protein to starch within the kernel. The limitation of nitrogen by the rust seems to result directly in the production of a softer grain of lighter color. In the hard wheats the proportion of yellow-berry kernels is increased by the leaf-rust infection, whereas in a soft wheat a more uniformly starchy kernel results.

The quality of soft wheats may not be seriously impaired by these effects of leaf rust, since low-strength flours of at least moderately low protein content are desirable in the production of crackers, pastry, and biscuits, for which the soft-wheat flours are primarily used.[5] The effect of a reduced protein-percentage content and an increased proportion of yellow-berry kernels, however, would constitute a serious impairment in the quality of hard red winter wheat, where high strength of flour and high protein content are desirable. At La Fayette, Ind., the marked effect of leaf rust on the quality of Fulhard wheat, a hard winter type, suggests that a similar effect may occur in the hard-winter-wheat region. This may occur especially in years when nitrogen is a limiting factor.

The extreme variation in the severity of leaf rust in different years is apparently an important contributing factor in the variability in the quality of the winter wheats from year to year. The development and use of winter-wheat varieties resistant to leaf rust would eliminate this factor as a cause of variability.

So far as known, neither agronomists nor physiologists have considered leaf rust a factor in the yellow-berry problem, although there has been marked diversity of opinion regarding the relative importance of the influences of heredity and of climatic and fertility conditions on the expression of yellow berry. Bolley (*1*) has ascribed the cause of yellow berry, as well as black point and other kernel diseases, to direct infection with fungi. Later workers, Headden (*7*) and Jones and Mitchell (*11*), have shown that yellow berry is clearly distinguishable from other kernel blemishes caused by fungous infection and that it may be controlled by cultural practice. It should be understood that leaf rust also may produce the yellow-berry condition but does so through its effect on the whole plant and not by direct infection of the grain.

The significance of leaf-rust infection of experimental plots has received little recognition in studies of the normal development of the wheat kernel. Most workers have failed to mention its presence or absence. Saunders (*18*) has described what apparently was a moderately severe leaf-rust epiphytotic, which was "rather bad" in his experimental plots 11 days before the grain ripened. However, he dismissed leaf rust as a significant factor in his chemical studies of the development of the kernels. This omission by investigators of the chemistry of kernel development undoubtedly is due to the very slight effect of leaf rust on the size and shape of the grain in contrast to the drastic effect of stem rust, which they could not disregard. A record of the occurrence of leaf rust might have helped to explain in many cases the great diversity of results in studies of normal development of the wheat kernel.

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[5] Samples of the wheats from the experiments reported in this paper have been experimentally milled, and baking studies on the flours are in progress.

SUMMARY

A study was made at La Fayette, Ind., in 1931, of the effects of a severe leaf-rust epiphytotic on the yield and physical characters of the grain and on the chemical composition of the grain and plants of (1) seven varieties of winter wheat of differing degrees of leaf-rust resistance, and (2) of one very susceptible variety infected with rust in four different degrees of severity. Very lightly rusted control plants were provided for comparison by frequently dusting the plants with sulphur, while variations in the dusting procedure provided a graded series of severities of rust infection.

In the very susceptible varieties, with one exception, reductions in yield of grain ranging from 14.8 to 28.4 percent were associated with heavy infections of leaf rust. The yield of Fulhard was not reduced, even though this variety was severely rusted. In most varieties the losses were approximately proportional to the severity of the rust. The yields of straw and grain were affected alike.

Approximately three-fourths of the grain losses caused by leaf rust resulted from a reduction in the number of kernels per head, and the remainder from a reduction in weight per kernel.

The most marked effect of leaf rust on the physical characters of the grain was a greatly increased proportion of yellow-berry or piebald kernels in the hard and semihard varieties, and the production of kernels more uniformly soft or starchy in the soft wheats. Even under almost maximum severity of rust at flowering time no shriveling of the kernels occurred. The test weight per bushel was slightly, though consistently, reduced by leaf rust.

The percentage of protein in the grain of susceptible varieties of both hard and soft winter wheats was very significantly reduced by severe leaf-rust infection. The effects were less pronounced in the more resistant varieties. The same was true of a susceptible variety when leaf rust was partly controlled.

In contrast to the grain, the combined culms and leaves of the rusted plants contained higher percentages of total nitrogen, and, in most cases, greater quantities of total nitrogen per tiller than did those of the control plants.

The starch percentage content of the mature grain varied inversely with the protein percentage, the lower-protein grain from rusted plants being higher in starch than was the grain from control plants. However, because of the reduced number and size of kernels, the total quantity of starch laid down per kernel and head was distinctly reduced by leaf rust.

Sucrose of the mature grain, although a minor constituent, was consistently reduced in concentration by leaf rust.

The culms and leaves of rusted plants at the nearly ripe stages contained lower percentages of both sucrose and reducing sugars than did the control plants.

The percentages of phosphorus and total ash of the grain were not appreciably affected by leaf rust.

Fultz selection, a highly resistant wheat, which was practically unaffected by leaf rust, showed no direct effect of sulphur dusting on the visible characters of the grain or on composition.

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INHERITANCE OF RESISTANCE TO LOOSE SMUT AND COVERED SMUT IN SOME OAT HYBRIDS[1]

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INTRODUCTION

In recent years several investigators have obtained extensive data on the inheritance of smut resistance in oat (*Avena*) hybrids. Crosses between oat varieties showing various types of resistance to the smuts were made, and the behavior of the hybrids in the F_2 and F_3, and, occasionally, in later generations was recorded. The published results have been reviewed in recent papers by Coffman et al. (*3*)[2] and Reed (*14*). The present paper deals with six oat crosses involving varieties that show different degrees of resistance to loose smut (*Ustilago avenae* (Pers.) Jens.) and covered smut (*U. levis* (Kell. and Sw.) Magn.). Data on the reaction of F_2 and F_3 plants are included.

Since recent investigations have demonstrated a high degree of specialization in both species of oat smuts, it is particularly important, in studies on the inheritance of smut resistance, not only to distinguish between the morphological species *Ustilago avenae* and *U. levis* but to recognize the fact that both species contain specialized races or physiologic forms characterized by differences in their capacity for infection. Reed (*7, 9, 11*) differentiated 9 distinct races of loose smut and 5 of covered smut. More recently, Reed and Stanton (*16*) have described a new race of covered smut distinguished by its capacity for infecting strains of the Fulghum oat. Reed (*15*) has also discovered that this race of smut is capable of infecting Black Mesdag, a variety exceptionally resistant to other known races of loose and covered smuts. In the studies herein described 3 distinct specialized smut races were used for inoculation, 2 belonging to *Ustilago avenae* and 1 to *U. levis*. These specialized races are designated as *U. avenae*-Missouri, *U. avenae*-Fulghum, and *U. levis*-Missouri. Their characteristics have been reported in the publications noted above.

MATERIALS AND METHODS

The six oat crosses studied were Monarch Selection × Black Mesdag, Richland × Fulghum, Richland × Markton, Markton × Iogold, Markton × Black Mesdag, and Cornellian × Markton. There were two hybrids of each varietal combination. The first-named variety was used as the female parent in all the crosses; there were no reciprocals. The crosses were made in the greenhouse

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[2] Reference is made by number (italic) to Literature Cited, p. 1082.

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at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C., in the spring of 1927. The parents were the strains of these varieties grown by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Detailed information as to the origin, economic value, and main characteristics of the varieties used as parents may be found in the literature on the registration and classification of oats (*4*, *17*, *19*, *20*, *21*, *22*). Black Mesdag (C.I.[3] no. 1877) and Markton (C.I. no. 2053) are of special interest because of their high degree of resistance to both species of smuts. Markton also has proved to be a valuable commercial variety in Washington, Oregon, Idaho, and western Montana (*22*). Cornellian (C.I. no. 1242) is a comparatively new, gray-kerneled variety, grown extensively in New York (*6*). Fulghum (C.I. no. 708) is widely distributed in the central and southern oat areas, and Iogold (C.I. no. 2329) and Richland (C.I. no. 787) are extensively grown in the Corn Belt States. Black Mesdag and Monarch Selection (of Etheridge) are black-kerneled varieties of little agronomic value.

Seed from the F_1 plants and from the parental strains was sent to the Brooklyn Botanic Garden, Brooklyn, N.Y., in the spring of 1928. A detailed study of the inheritance of smut resistance in these hybrids was conducted in 1928, 1929, 1930, and 1931. Three sets of seed were taken from each F_1 plant of the different crosses. One set was inoculated with *Ustilago avenae*-Missouri, the second with *U. avenae*-Fulghum, and the third with *U. levis*-Missouri. The three sets of F_2 plants were grown in 1928, together with the parental varieties similarly inoculated. In 1929 there were grown additional F_2 plants of some of the crosses inoculated with *U. avenae*-Missouri and *U. levis*-Missouri.

The experiments with the F_3 generation of the hybrids were conducted under field conditions in 1929, 1930, and 1931. Usually, two sets of seed were taken from each F_2 plant, one set being inoculated with *Ustilago avenae*-Missouri and the other with *U. levis*-Missouri. In one of the crosses, however, one set of the F_3 progenies was inoculated with *U. avenae*-Fulghum instead of with *U. levis*-Missouri. The F_3 progenies were grown together with the parental varieties similarly inoculated.

The methods of inoculating and germinating the seed have been fully described by Reed (*14*). The usual procedure was to remove the hulls, inoculate the seed with dry chlamydospores, and allow them to germinate in sand with a water content of approximately 20 percent at a temperature of about 20° C. When the seedlings were 1 or 2 inches high they were transplanted to the field.

The profound influence of environmental factors on the infection of oats with the smuts is recognized by careful investigators. The low percentages of infection reported by a few workers may be accounted for by neglect to control soil moisture, soil temperature, and other conditions. The presence of hulls, especially in connection with certain external factors, has an important influence on the number of smutted plants. In the present studies the influence of such factors was largely eliminated, and the results obtained must be accounted for on the basis of inherited factors governing resistance.

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[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

EXPERIMENTAL DATA

F$_2$ PLANTS

Table 1 presents the summarized data on the reaction of the inoculated F$_2$ plants of the six hybrids and the data on the inoculated parental varieties grown with the F$_2$ plants and the F$_3$ progenies.

In general, the data in table 1 conform to expectation, but there are some exceptions that should be pointed out. The combination Monarch Selection × Black Mesdag is of interest. Monarch Selection is very susceptible to *Ustilago avenae*-Missouri but highly resistant to *U. levis*-Missouri, whereas Black Mesdag is highly resistant to both smuts. The F$_2$ results from this cross, as shown in table 1, might be interpreted as indicating that smut resistance is dominant and susceptibility recessive and that segregation for smut resistance has occurred on the basis of a 3:1 ratio.

The only exception to the expected in the F$_2$ behavior of the Markton × Black Mesdag cross, a combination involving the two most highly resistant varieties known, is the occurrence of 1 smutted plant in 69 tested with *Ustilago avenae*-Missouri.

Only 3 of 69 F$_2$ plants of the Markton × Iogold cross were infected with *Ustilago avenae*-Missouri, although 30.1 percent of the Iogold plants, the susceptible parent, were smutted. No infection whatever was obtained with *U. avenae*-Fulghum, as was to be expected. An inconsistent result was obtained with *U. levis*-Missouri in that each parent showed some slight infection, whereas the F$_2$ plants were entirely free from smut.

TABLE 1. *Reaction to 3 races of smut of inoculated F$_2$ plants of 6 oat crosses and their parental varieties, grown at the Brooklyn Botanic Garden, Brooklyn, N.Y., 1928 and 1929*

Parental variety or cross	Hybrid no. assigned by— Division of Cereal Crops and Diseases	Hybrid no. assigned by— Brooklyn Botanic Garden	Plants inoculated with— *Ustilago avenae*-Missouri: Plants grown	*Ustilago avenae*-Missouri: Infected		*Ustilago avenae*-Fulghum: Plants grown	*Ustilago avenae*-Fulghum: Infected		*Ustilago levis*-Missouri: Plants grown	*Ustilago levis*-Missouri: Infected	
			No	*No*	*Pct.*	*No*	*No*	*Pct*	*No*	*No*	*Pct*
Monarch Selection (936)[a]			95	89	93.7	30	15	50.0	92	0	0.0
Monarch Selection (936) ×	275-1	37	28	5	17.9	28	6	21.4	72	0	0
Black Mesdag (926)	275-2	38	45	17	37.8	27	3	11.1	30	0	0
Black Mesdag (926)			117	0	.0	25	0	.0	124	0	0
Black Mesdag (927)			141	0	.0	28	0	.0	146	0	0
Markton (934) × Black Mesdag (927) F$_2$	2753-1	46	27	1	3.7	30	0	0	79	0	0
	2753-2	47	42	0	.0	25	0	.0	44	0	.0
Markton (934)			152	0	0	25	0	.0	148	0	0
Markton (933)			154	0	.0	16	0	0	153	1	7
Markton (933) × Iogold (930)	2737-1	43	25	1	4.0	25	0	0	78	0	0
F$_2$	2737-2	44	44	2	4.5	26	0	.0	57	0	0
Iogold (930)			153	46	30.1	26	0	.0	147	6	4.1
Cornellian (928)			169	90	53.3	23	0	0	172	0	0
Cornellian (928) × Markton	2754-1	48	27	2	7.4	20	0	.0	70	0	.0
(935) F$_2$	2754-2	49	40	4	10.0	27	0	.0	45	2	4.4
Markton (935)			175	0	.0	26	0	0	173	0	.0
Markton (932)			183	0	0	29	0	0	151	0	.0
Richland (938) × Markton	2712-1	41	28	0	0	28	0	.0	78	2	2.6
(932) F$_2$	2712-2	42	39	1	2.6	26	0	0	49	3	6.1
Richland (938)			110	9	8.2	22	0	.0	109	12	11.0
Richland (937)			135	6	4.4	119	1	.8	13	9	69.2
Richland (937) × Fulghum	2710-1	39	48	16	33.3	51	16	31.4	21	7	33.3
(929) F$_2$	2710-2	40	26	9	34.6	66	23	34.8	27	10	37.0
Fulghum (929)			159	6	3.8	113	89	78.8	24	2	8.3

[a] Numbers in parentheses indicate special seed numbers assigned to strains of these varieties inoculated and observed by G. M. Reed.

Only 6 of 67 F_2 plants of the Cornellian × Markton cross were infected with *Ustilago avenae*-Missouri, yet the susceptible parent variety showed an average infection of 53.3 percent, there being no infection whatever in the Markton parent. Neither of these parent varieties reacted to *U. levis*-Missouri, but 2 of the F_2 plants were infected with this smut.

The reaction of the F_2 plants of the Richland × Markton cross to the races of smut corresponded rather closely to that of the two parental varieties.

In the case of each of the three races of smut, approximately one-third of the inoculated F_2 plants of the Richland × Fulghum cross were infected, and no marked difference was obtained in the behavior of the two hybrids. However, it will be seen from table 1 that an approximate average of 34 percent of infection was obtained with *U. avenae*-Missouri, yet the parents, Richland and Fulghum, showed only 4.4 and 3.8 percent of infection, respectively.

F_3 PROGENIES

As previously stated, two sets of seed were usually taken from each F_2 plant, one set being inoculated with *Ustilago avenae*-Missouri, and the other with *U. levis*-Missouri. With the exception of the Richland × Fulghum cross, no F_3 progenies inoculated with *U. avenae*-Fulghum were grown. On the basis of the treatment of the F_2 plants three groups of F_3 progenies were available for study: (1) Progenies descended from F_2 plants inoculated with *U. avenae*-Missouri, (2) progenies descended from F_2 plants inoculated with *U. avenae*-Fulghum, and (3) progenies descended from F_2 plants inoculated with *U. levis*-Missouri.

Table 2 presents the results obtained in F_3 after inoculation with all races of smut. For convenience and comparison, the F_3 progenies are grouped into three main classes: (1) Resistant, in which no infected plants were observed; (2) segregating, in which from 1 to 50 percent of the plants were infected; and (3) susceptible, which contained more than 50 percent of smutted individuals. The percentages of infection in the inoculated parental varieties and in the F_2 plants and F_3 progenies are shown.

REACTION TO USTILAGO AVENAE-MISSOURI

As shown in table 2, the reaction of the F_3 progenies of hybrids 37 and 38 of Monarch Selection (very susceptible to *Ustilago avenae*-Missouri) × Black Mesdag (resistant to *U. avenae*-Missouri) is in accord with genetic expectation. The results obtained from this cross in F_3 confirm the interpretation indicated in F_2 that smut resistance is dominant and susceptibility recessive, and that segregation for the inheritance of smut resistance has occurred on the basis of a 3:1 ratio. On the basis of the data obtained it might be expected that there would be no susceptible progenies in the first group of F_3 progenies descended from F_2 plants tested with *U. avenae*-Missouri, since the F_2 parent plants susceptible to *U. avenae*-Missouri should have been eliminated. It is possible, however, that the 4 highly susceptible progenies were descendants from susceptible F_2 plants that had escaped infection. Assuming that this is the correct explanation, the remaining 45 progenies of this group show a fairly close approximation of a ratio of 1:2, 16 progenies being resistant and 29 segregating.

TABLE 2.—*Reaction to 3 races of smut of inoculated F_3 progenies of 6 oat crosses and their parental varieties, grown at the Brooklyn Botanic Garden, Brooklyn, N.Y., 1929, 1930, and 1931*

Smut species and races used in inoculation	Infection in parent varieties		Infection in F_2 plants	F_3 progenies inoculated with—											
				Ustilago avenae-Missouri				*Ustilago levis*-Missouri				*Ustilago avenae*-Fulghum			
	♀	♂		Resistant	Segregating	Susceptible	Infected	Resistant	Segregating	Susceptible	Infected	Resistant	Segregating	Susceptible	Infected
	Percent	*Percent*	*Percent*	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*
MONARCH SELECTION × BLACK MESDAG (HYBRIDS 37–38)															
U. avenae-Missouri	93.7	0.0	30.1	16	29	4	67.3	29	19	0	39.6	----	----	----	----
U. avenae-Fulghum	50.0	.0	16.4	17	26	3	63.0	28	17	0	37.8	----	----	----	----
U. levis-Missouri	.0	.0	0	32	60	28	73.3	71	39	1	36.0	----	----	----	----
MARKTON × BLACK MESDAG (HYBRIDS 46–47)															
U. avenae-Missouri	0.0	0.0	1.4	43	11	0	20.4	33	4	0	10.8	----	----	----	----
U. avenae-Fulghum	.0	.0	0	30	9	1	25.0	31	9	0	22.5	----	----	----	----
U. levis-Missouri	0	.0	.0	60	18	0	23.1	64	12	0	15.8	----	----	----	----
MARKTON × IOGOLD (HYBRIDS 43–44)															
U. avenae-Missouri	0.0	30.1	4.3	36	20	0	35.7	34	3	0	8.1	----	----	----	----
U. avenae-Fulghum	.0	0	0	28	12	0	30.0	39	1	0	2.5	----	----	----	----
U. levis-Missouri	7	4.1	.0	49	29	0	37.2	68	7	0	9.3	----	----	----	----
CORNELLIAN × MARKTON (HYBRIDS 48–49)															
U. avenae-Missouri	53.3	0.0	9.0	19	21	1	53.7	23	5	0	17.9	----	----	----	----
U. avenae-Fulghum	.0	.0	0	24	13	2	38.5	32	6	0	15.8	----	----	----	----
U. levis-Missouri	.0	0	1.7	43	29	1	41.1	41	13	0	24.1	----	----	----	----
RICHLAND × MARKTON (HYBRIDS 41–42)															
U. avenae-Missouri	8.2	0.0	1.5	9	13	0	59.1	7	4	0	36.4	----	----	----	----
U. avenae-Fulghum	.0	.0	.0	18	8	1	33.3	19	5	0	20.8	----	----	----	----
U. levis-Missouri	11.0	0	3.8	45	17	0	27.4	44	15	0	25.4	----	----	----	----
RICHLAND × FULGHUM (HYBRIDS 39–40)															
U. avenae-Missouri	4.4	3.8	33.8	7	20	2	75.9	----	----	----	----	0	23	2	100.0
U. avenae-Fulghum	8	78.8	33.3	18	39	8	72.3	----	----	----	----	8	19	0	70.4
U. levis-Missouri	69.2	8.3	35.4	5	14	1	75.0	----	----	----	----	4	16	0	80.0

The results obtained in the second group of F_3 progenies (descended from F_2 plants tested with *Ustilago avenae*-Fulghum) of Monarch Selection × Black Mesdag indicate some correspondence in the reaction of the hybrids to the two races of loose smut used in these experiments. If the reaction of the different progenies to these two smuts is not correlated, a much larger number of susceptible families in the group inoculated with *U. avenae*-Missouri would be expected. It is unfortunate that no F_3 data are available from progenies of plant material that had been inoculated in F_2 with *U. avenae*-Fulghum. Such data might indicate the extent of the similarity in the behavior of the hybrids toward the two smuts.

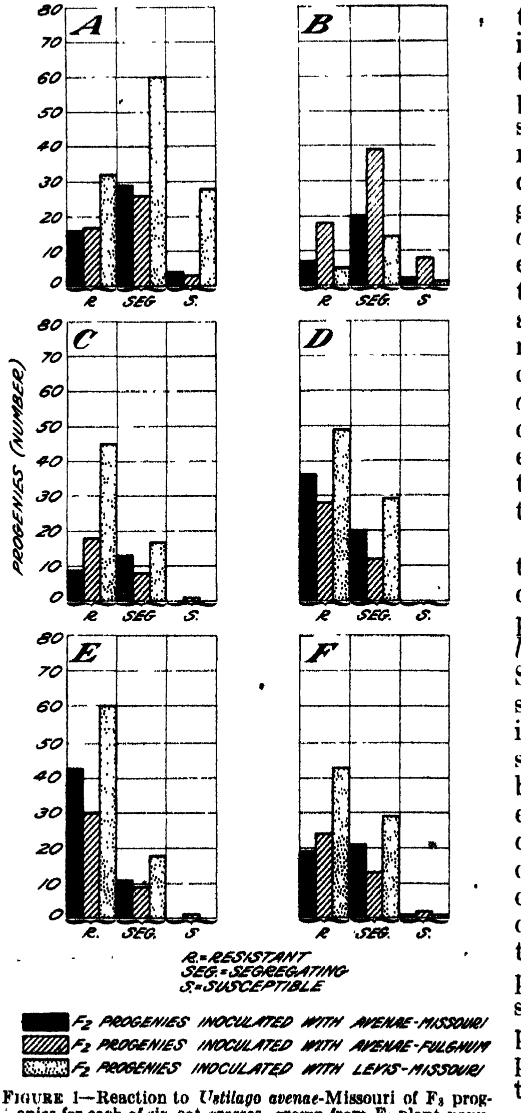

FIGURE 1—Reaction to *Ustilago avenae*-Missouri of F_3 progenies for each of six oat crosses, grown from F_2 plant populations that had been inoculated with *U. avenae*-Missouri, *U. avenae*-Fulghum, and *U. levis*-Missouri: *A*, Hybrids 37 and 38; *B*, hybrids 39 and 40; *C*, hybrids 41 and 42; *D*, hybrids 43 and 44; *E*, hybrids 46 and 47; *F*, hybrids 48 and 49.

The 120 F_3 progenies of the third group of F_3 progenies descended from F_2 plants tested with *Ustilago levis*-Missouri of Monarch Selection × Black Mesdag showed a ratio of approximately 1 resistant to 2 segregating to 1 susceptible. No F_2 plants had been eliminated, and consequently F_3 progenies of the different classes might be expected. Of the 28 susceptible progenies obtained, 1 showed 64.2 percent of infection, 2 showed from 70.1 to 80 percent, 5 from 80.1 to 90 percent, and 20 from 90.1 to 100 percent.

The occurrence of smut in the F_3 progenies of hybrids 46 and 47 (Markton × Black Mesdag) was unexpected. As shown in table 2, a large number of the progenies were resistant. However, approximately one-fourth of the progenies contained smutted individuals. One showed as much as 56.2 percent of infection, an interesting result which is difficult of explanation. The behavior of this cross is treated more fully on page 1081.

In F_3 progenies of hybrids 43 and 44 (Markton × Iogold) there were a large number of resistant progenies in each F_2 smut group, but no progeny was classified as susceptible.

Hybrids 48 and 49 (Cornellian × Markton) involve a cross between a resistant variety and one that is rather susceptible, the smut in the Cornellian plants ranging from 27.2 to 72 percent in the different tests. As shown in table 2, a predominance of resistant progenies was obtained in the F_3. Furthermore, only four progenies were classified as susceptible, and the highest percentage of infection obtained in any of these was less than 70. The data for the F_2 and the F_3 generations do not indicate any simple dominance and segregation.

Hybrids 41 and 42 (Richland × Markton) involve a cross between a resistant variety and one that shows a very low percentage of infection. In the F_2 generation 67 plants were inoculated and only 1 was infected. Only 1 F_3 progeny was classified as susceptible, 70.5 percent of the individuals being infected. Most of the progenies were entirely resistant.

The Richland and Fulghum parents of hybrids 39 and 40 were slightly susceptible to *Ustilago avenae*-Missouri, both showing less than 5 percent of infection. In contrast to the two parental varieties, a comparatively large number of the F_2 hybrids were infected. In each of the three groups of F_3 progenies there were 2 or 3 times as many segregating progenies as there were resistant ones. Susceptible progenies also were found in each group, most of the groups containing a high proportion of infected individuals.

The number of infected F_3 progenies in each of the six crosses inoculated with *Ustilago avenae*-Missouri is shown in figure 1.

REACTION TO USTILAGO LEVIS-MISSOURI

As has already been shown, the F_2 generations of the six crosses were inoculated and tested with *Ustilago levis*-Missouri, and F_3 progenies of five of the six crosses were tested with the same race of smut. Table 2 shows that no experiments with *U. levis*-Missouri were conducted with F_3 progenies of hybrids 39 and 40 (Richland × Fulghum).

Many F_3 progenies in the *Ustilago levis*-Missouri series contained smutted individuals, although both parental varieties of the crosses were resistant (table 2). Hybrids 37 and 38 (Monarch Selection × Black Mesdag) included 128 resistant F_3 progenies, 75 segregating, and 1 that was classed as susceptible. This susceptible progeny contained 22 plants, of which 13 (59 percent) were smutted. The 75 segregating progenies contained 1,492 plants, of which 205 were smutted. Hybrids 46 and 47 (Markton × Black Mesdag) included 128 resistant progenies, 25 segregating, and none that was susceptible; the 25 segregating progenies included 493 plants, of which 50 were infected. Hybrids 48 and 49 (Cornellian × Markton) included 96 resistant progenies, 24 segregating, and none that was susceptible; the 24 segregating progenies included 479 plants, of which 43 were infected. In nearly all these segregating progenies the number of infected plants was small—usually but 1 or 2 being observed.

The number of infected F_3 progenies in each of five crosses inoculated with *Ustilago levis*-Missouri is shown in figure 2.

REACTION TO USTILAGO AVENAE-FULGHUM

Studies were made with the Fulghum race of *Ustilago avenae* only on the F_3 plants of hybrids 39 and 40 (Richland × Fulghum). In the various experiments, Fulghum showed 78.7 percent of infection with this race of smut, whereas Richland was nearly free from infection. No resistant F_3 progeny was obtained in the first group (descended from F_2 plants tested with *Ustilago avenae*-Missouri). Very few susceptible F_3 progenies were obtained in any of the groups; several might have been expected in the first and third groups, unless there was some correspondence in the inheritance of resistance to all three races of smut. Comparatively high susceptibility in the F_2 generation to all three races of smut was associated with comparatively few F_3 progenies susceptible to *U. avenae*-Fulghum.

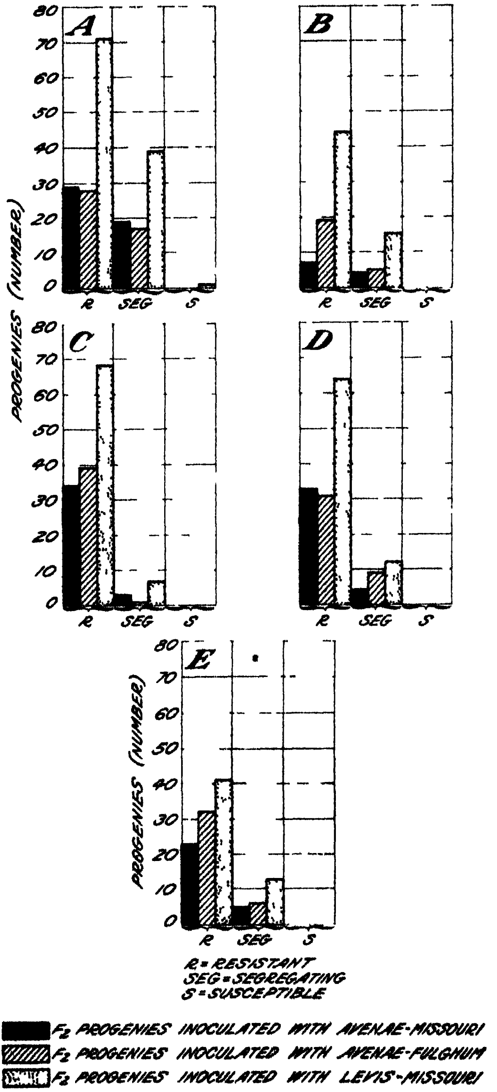

FIGURE 2 Reaction to *Ustilago levis*-Missouri of F_3 progenies for each of five oat crosses, grown from F_2 plant populations that had been inoculated with *U avenae*-Missouri, *U avenae*-Fulghum, and *U levis*-Missouri *A*, Hybrids 37 and 38; *B*, hybrids 41 and 42, *C*, hybrids 43 and 44, *D*, hybrids 46 and 47, *E*, hybrids 48 and 49.

DISCUSSION

The results obtained from inoculating F plants of hybrids 37 and 38 (Monarch Selection × Black Mesdag) with *Ustilago avenae*-Missouri show a simple dominance for resistance to this smut. These results are in accord with those reported by Reed (*8, 10, 12, 13*) in his studies on smut resistance in hybrids of Hull-less × Black Mesdag, Early Gothland × Victor, and Early Gothland × Monarch. In these crosses one parent was susceptible to the particular races of smut used and the other was resistant. The results showed a simple dominance for the inheritance of resistance, segregation occurring on the basis of a 3:1 ratio in F_2. The results obtained in F_3 were in harmony with the interpretation.

The presence of segregating progenies in F_3 in certain crosses in which no infection was expected is doubtless connected with the behavior of certain oat varieties. Occasionally an infected plant is found in a row of Markton when this variety is inoculated with *Ustilago levis*-Missouri. Fulghum frequently shows a small percentage of infection with both *U. avenae*-Missouri and *U. levis*-Missouri. Iogold and Cornellian show a comparatively high percentage of smutted plants. It has, however, been very difficult to approach 100 percent of infection in either of these varieties.

The further question arises as to the extent to which the fungus develops in individuals of such varieties as Markton, Fulghum, and Iogold, the plants of which finally appear normal. Kolk (*5*) has shown that, in the resistant Black Mesdag, *Ustilago avenae*-Missouri penetrates and ramifies more or less extensively throughout the coleoptile. Apparently, however, it does not gain entrance into the developing embryonic tissue.

Bayles and Coffman (*1*), in studies of the effect on germination of removing the hulls from seed of certain oat varieties, report a reduction of 5.1 percent in seedling emergence in the Markton variety when seed with hulls removed was inoculated with smut. A similar reduction was observed in the susceptible varieties Early Champion and Sixty-Day. Stanton et al. (*18*), reporting studies on the effect of removing the hull on seedling emergence in oat varieties, showed that although Markton gave no apparent indication of smut infection, yet seedling emergence was reduced by 2.9 percent through inoculation with smut. It was concluded from this evidence that Markton is not completely free from infection by the smut organism.

The occurrence of marked smut infection in F_3 progenies of the Markton × Black Mesdag cross is difficult to explain. It is possible that complementary factors may account for the reduction, or apparent breaking down, of resistance in the F_3 progenies of this cross. Briggs (*2*) has observed the occurrence of smutted plants in some of his resistant progenies of hybrids between Hard Federation and Hussar wheat varieties. No smut appeared in the F_2 and F_3, but in the F_4 and subsequent generations slightly susceptible families were obtained. Briggs interprets his results on the basis of modifying factors.

In the Markton × Black Mesdag cross, 25 percent of the F_3 progenies descended from F_2 plants inoculated with *Ustilago avenae*-Fulghum were infected (table 2). The occurrence of smut infection in so many progenies is hardly analogous to the appearance of a few smutted individuals in F_4 and subsequent generations of certain wheat crosses reported by Briggs (*2*). As a consequence, these results cannot be interpreted on the basis of modifying factors. It is probable that complementary factors may offer a more satisfactory basis for interpretation, since so high a percentage of the F_3 progenies were smutted. Both Markton and Black Mesdag may carry complementary factors for susceptibility, which, when brought together through hybridization, may produce smutted plants.

Another interesting result is the relation between the number of resistant, segregating, and susceptible F_3 progenies. In the *Ustilago levis*-Missouri series there is a preponderance of resistant progenies in all five hybrids. The same is true in the *U. avenae*-Missouri series of hybrids 41 and 42, 43 and 44, 46 and 47, and 48 and 49. Of course these results would be expected to a large extent on the basis of the behavior of the parental varieties, since none of them showed anything like 100 percent of infection.

The loss of the susceptible individuals in these hybrids introduces a difficulty in the complete analysis of smut inheritance. The smutted plants being destroyed, their subsequent generations cannot be grown.

SUMMARY

This paper presents results obtained with six oat hybrids, involving crosses between varieties that differ in their behavior to certain races of the oat smuts.

F_2 plants of all these crosses, inoculated with *Ustilago avenae*-Missouri, *U. levis*-Missouri, and *U. avenae*-Fulghum, were grown and studied. Progenies of all 6 crosses inoculated with *U. avenae*-Missouri, progenies of 5 crosses inoculated with *U. levis*-Missouri, and progenies of 1 cross inoculated with *U. avenae*-Fulghum also were grown in F_3, and their reaction to these smuts was recorded.

The data for hybrids 37 and 38 (Monarch Selection × Black Mesdag) inoculated with *Ustilago avenae*-Missouri indicate that smut resistance is inherited on the basis of a 3:1 ratio.

A most noteworthy feature is the occurrence of considerable smut in some F_3 progenies of hybrids descended from entirely resistant parents.

In most of the hybrids completely resistant progenies predominated over segregating progenies; there were very few susceptible progenies recorded.

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TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT[1]

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INTRODUCTION

In 1930 the writer (*3*)[2] described a procedure whereby it seemed possible to compare alfalfa (*Medicago sativa* L.) varieties in regard to their resistance to bacterial wilt, caused by *Phytomonas insidiosa* McCulloch, in the space of a single year, thereby avoiding the delay and climatic hazards inevitable in a field test in infested soil. The procedure described permitted not only the comparison of the degree of resistance in varieties but also the selection of resistant plants from which resistant strains may be developed. During 3 years the writer has used this method in comparing resistance in plants from a considerable number of seed lots of alfalfa. Modifications of the procedure, which would permit testing larger numbers of plants with the facilities available for the purpose at Madison, Wis., have been tested. The development of the parasitic bacteria in resistant and in susceptible plants has been compared. The present paper is a report on the progress of the work thus outlined.

In 1930 Peltier and Tysdal (*7*) published results from a single but extensive trial of the method described by the writer (*3*). In 1933 Peltier (*5*) published additional data of a similar character. All of these results are substantially in agreement with those presented here. In 1932 Peltier and Schroeder (*6*) in a study of the nature of resistance of alfalfa to wilt reached the conclusion that in the material studied resistance was in the main associated with morphological features of the root. The writer is unable to corroborate this conclusion. These results by Peltier and his associates will be discussed later in this paper.

COMPARISON OF VARIETIES FOR RESISTANCE

The procedure in comparing seed lots for resistance has been little altered from that originally suggested (*3*). The seed is planted in flats in the greenhouse, preferably in November, but when necessary as late as the middle of January. When the seedlings are about a month old they are transplanted, usually into beds where they are spaced about 1½ inches apart in rows 3 inches apart. As early in May as possible the seedlings are washed from the soil, inoculated, and transplanted to the field, where they are spaced 4 inches apart in rows 10 inches apart. In 1930, inoculation was made by scraping opposite sides of the root lightly with a knife under a bacterial suspension from several pure cultures of different origin. In the 2 following years incision was made through the bark with a sharp knife under a bacterial suspension instead of scraping. A second inoculation was

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[2] Reference is made by number (italic) to Literature Cited, p. 1098

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made of the plants in the entire plot in 1930 and of a part of those in the plot in 1931 in early August in the following manner. After all plants showing disease symptoms were pulled, earth was removed from around the crowns of those remaining, and a thin slice of diseased tissue from one of the infected plants was inserted in an incision through the bark of the taproot. The earth was then replaced about the crowns and the ground was well watered. This second inoculation cannot be made rapidly, and therefore it was not carried out with plants from the seed lots from Turkistan in 1931 nor was it attempted at all in 1932. Three cuttings have usually been made in the early bloom stage.

After active growth had ceased in the fall, the plants were dug for examination and divided into two classes, namely, those infected and those uninfected. Evidence of infection was first sought by cutting the main root of each plant at about 6 inches below the crown. If no discoloration characteristic of the disease could be found in any root at this distance from the crown, the scar of the wound made at inoculation was examined. If no discoloration was found here after making such incision as seemed necessary, the plant was classed as uninfected. Such an examination will not always reveal local infections near the crown, and therefore occasional plants judged uninfected and placed in the greenhouse have subsequently developed disease; but these occasional errors in classification are preferable to a more thorough examination of the plant which destroys its immediate usefulness in seed production.

The method outlined may be varied by starting the plants at different times in the year and inoculating at different stages of development in the greenhouse as well as in the field. Several variations which might be serviceable if successful have been tried. Chief among these are the following:

(1) Seed sown in the field in early spring.
  (*a*) Inoculation at transplanting to rows in the field, as with greenhouse-grown plants; as soon as seedlings are large enough to inoculate conveniently through wounds in the roots.
  (*b*) Inoculation at transplanting to the greenhouse in which the plants are grown through the summer.
  (*c*) Inoculation of spaced plants without transplanting.
(2) Seed sown in the field in late July or August; inoculation at transplanting to the greenhouse in October.

The first variation was tried extensively in 1930. Owing in part to a thick stand, the plants did not attain sufficient size for transplanting until late June, and transplanting extended into early July, when the temperature was high. While water was freely used at transplanting and subsequently, the plants did not make rapid growth during the entire summer, though few died. Infection was less than by the routine method, especially in the more resistant class. The period from inoculation to the end of the summer appeared much too short for advanced development of the disease in infected plants. The procedure gave useful comparisons between seed lots, but it did not result in the infection of nearly so many plants as did the routine method. It has not been tried further.

The second variation was likewise tried in 1930 in the hope of extending the growing season somewhat and thus obtaining a more thorough elimination of the susceptible plants. Seemingly excellent conditions for infection were provided by shading the greenhouse so that the temperature did not rise above 30° C. and by keeping the soil well watered. Nevertheless, infection was even less than in plants transplanted to the field at the time the greenhouse experiment was begun. The method appeared useless and was not tried further. In general, field-grown seedlings transplanted at any other time than in late autumn make less rapid recovery and subsequent growth than greenhouse-grown seedlings.

The inoculation of spaced seedlings grown in the field by digging around the plants and applying a bacterial suspension to a wound made in the root or by inserting bits of diseased tissue in the root has invariably proved a highly effective method in securing infection. In 1931 it was tried extensively. The disadvantages of this method are the amount of labor involved and the fact that in this latitude seedlings from the spring sowing do not attain sufficient size for inoculation until the growing season is far advanced. Thus when winter comes the disease has not progressed far, especially in the more resistant plants. It should be noted that inoculation by fragments of diseased tissue dipped in bacterial suspension and inserted in slits in the bark of the roots has given slightly higher infection than any other method tried. Tables of the results of this method as compared with results obtained by the use of several pure cultures are omitted for the sake of brevity.

Where seedlings grown in the field in late summer and early autumn were inoculated upon being transplanted to the greenhouse, it seemed that inoculation would be made into the most susceptible tissue the plant would produce under field conditions and that in consequence disease development would be rapid. In a single trial made with about 900 plants from 12 seed samples the anticipated high infection was obtained, an infection approximately equal to that obtained in the field from the inoculation of greenhouse seedlings. The method has not been used further because it requires much greenhouse space.

TABLE 1.—*Comparative test of alfalfa varieties and seed lots for resistance to bacterial wilt, 1930*

[Inoculation was made with pure culture at transplanting in May and by use of diseased tissue in August]

Origin or variety	Seed lot no.[a]	Plants inoculated		Origin or variety	Seed lot no.[a]	Plants inoculated	
		Total	Uninfected			Total	Uninfected
		Number	*Percent*			*Number*	*Percent*
Arizona	1289	217	1.5	Montana	1300	218	2
Argentine	1332	201	1.5	Peruvian	1290	219	0
Baltic	597	181	1	Ladak	1334	233	30
Cossack	1260	145	1.6	South Dakota	875	216	4
Grimm	999	137	1	Do	1173	228	2
Do	1335	195	1	Turkistan	1356	149	24
Kansas (Wichita)	1224	302	0	Do	84371 [b]P I	118	44
Kansas (Derby)	1225	209	9.5				
Do	1301	166	5	Utah	1157	158	3
Do	1302	194	1	Do	1219	134	0
Do	1303	202	2.5	Do	1245	225	.5
Do	1304	253	3				

[a] Identification number used at the Wisconsin Experiment Station.

[b] Identification number of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U.S. Department of Agriculture.

From this survey of possible variations in the methods for determining resistance in 1 year, it appears that the schedule originally suggested best combines convenience and effectiveness. The first extended comparison of seed lots of alfalfa by this routine method for determining wilt resistance was made in 1930. The seed samples used were largely American regional strains contributed by Prof. L. F. Graber, of the Wisconsin Agricultural Experiment Station. The results of the 1930 trials are presented in table 1. Very few plants from the American strains remained uninfected, whereas the two seed samples from Turkistan and the one from Ladak were strikingly distinguished by the comparatively large number of uninfected plants.

In 1931 some of the seed samples collected by H. L. Westover in Turkistan in 1929 and in Spain and northern Africa in 1930, together with a few miscellaneous samples of diverse origin, were tested. The plant populations from seed of undoubted Turkistan origin were sharply distinguished from all others by greater resistance. The results are summarized in table 2. A few additional samples from the same regions were compared in 1932 with the same result. This outcome appears to accord with the very extensive results obtained by Peltier and Tysdal (*7*) and by Peltier (*5*). The percentage of uninfected plants in the results cited is usually higher than that shown here. Such difference in the susceptible varieties may be due to the fact that in this class of plants a second inoculation was made by the writer, whereas in obtaining the results cited, but a single inoculation was made.

TABLE 2.—*Resistance to bacterial wilt in alfalfa from foreign sources after artificial inoculations in 1931–32*

Source	Seed lots	Plants inoculated			
		Number	Percentage uninfected		
			Highest	Lowest	Average
	Number		*Percent*	*Percent*	*Percent*
Turkistan.					
Samarkand	4	577	80	33	55
Tashkent	11	1,926	80	23	50
Ferghana	2	291	52	43	47
Khiva	1	59			54
Bukhara	1	108			69
Manchuria	1	63			0
China	4	390			3
Spain	45	4,873	1	0	.3
Portugal	4	500	1	0	.2
Northern Africa	33	4,203	1	0	.5
South America	4	491			.2

Alfalfa, then, falls into two distinct major classes with respect to resistance: A resistant class consisting of or derived more or less directly from Turkistan or Ladak, and a susceptible class comprising alfalfa from all other sources tested thus far. The result may be stated in another way. Resistance to bacterial wilt appears to be a characteristic of alfalfa in the region, or at least in much of the region, credited as being its place of origin. The boundaries of the region in which high resistance is a uniform characteristic are not precisely delimited by the data at hand. In the dissemination of the plant from this region of origin and adaptation to other regions where it is now grown the resistant character appears to have been largely lost, whatever habit of growth the plant has assumed in its new environment. That the disease occurs in Turkistan is attested by the fact noted previously,[3] that the bacteria causing it were recovered from two plants collected by Westover at a small village north of Bukhara. However, it is unlikely that the disease is abundant or conspicuous in this region at present, as Westover has stated to the writer that he recognized the disease only at this location, in a field that had been excessively watered. Thus it appears possible that the parasite has been constantly associated with the plant in Turkistan, enforcing a selection for resistance in that region, and that the parasite has not followed the plant elsewhere to continue that selection until a comparatively recent introduction into the United States; but facts are not at hand whereby this suggestion may be confirmed.

COMPARISON OF INDIVIDUAL PLANTS FOR RESISTANCE

Whatever the historical background of the present regional separation of the resistant and susceptible varieties of alfalfa, the present task is production by artificial selection of resistant varieties suited to regional needs. Two sources of material are available for use. (1) Strains of highly resistant alfalfa from central Asia and (2) individual resistant plants that may be found in present commercial varieties. If individual plants from susceptible varieties are chosen for the building of varieties, much use of artificial inoculation for the elimination of susceptible plants from populations will be required. Therefore, a critical appraisal of the accuracy and limitations of artificial inoculation as a tool both in comparison of varieties and in selection should be made.

That a test carried out in the field where conditions differ from year to year will give identical results is hardly to be expected; and in fact it has not always given closely similar results in the same season from transplantings made but a short space of time apart. Large lots of seedlings have been divided and transplanted at several dates through May and June, but differences in the results of these experiments have not been greater than differences that have occurred from inoculations made a few days apart. In a few Turkistan seed samples differences in the percentages of uninfected plants have amounted to 30 percent in exceptional cases. In susceptible varieties differences have not been so great, due perhaps in part to the second inoculation. Such differences were not only perplexing in the plots at Madison, but became even more so when an attempt was made to duplicate some of the work elsewhere. For instance, in 1930 some of the seed samples numbered in table 1 were grown by C. O. Grandfield at Manhattan, Kans., in the greenhouse, and inoculated at transplanting with the same cultures as at Madison. At Manhattan the percentage of uninfected plants was determined by Grandfield in the autumn. On the whole, the percentage of uninfected plants was greater at Manhattan, probably because they were inoculated but once, but this result was not consistent in all the lots. For instance, one sample gave 25 percent of uninfected plants at Manhattan and but 2.5

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[3] JONES, F R BACTERIAL WILT OF ALFALFA IN TURKISTAN U S Dept Agr Bur Plant Indus Plant Disease Rptr. 14 125 1930 [Mimeographed]

percent at Madison, and a sample giving 7 percent of uninfected plants at Madison gave but 2.5 percent at Manhattan.

A study of planting and climatic data has been made in an effort to explain these differences; but thus far no fact or set of facts has been found with which the differences can be correlated. That the method of inoculation is usually sufficiently effective to prevent many plants from escaping infection, even though they escape wounding, was shown in 1931, when groups of 10 plants were set unwounded and uninoculated at intervals in the plot so that difference in growth between diseased and healthy plants might be observed. At the end of the season all these groups of uninoculated plants were found infected almost as severely as the inoculated plants, except in the final transplanting made early in June. Emphasis upon these irregularities in the results of inoculation should not obscure the fact that most repeated trials are closely consistent in result. A second inoculation of plants that have not shown evidence of disease in the foliage by the end of July appears to make results more consistent than does a single inoculation. However, this biological test, carried out in the field under varying climatic conditions, has not been and perhaps cannot be standardized to produce precise comparisons.

Thus far the discussion has been confined to the consistency of the test in defining the resistance of plant populations. In the selection of resistant plants it is even more important to know how accurately the test defines or can be adapted to define the resistance of individual plants. At the conclusion of a test in autumn some plants are found uninfected, others with varying degrees of disease development. The questions arise whether any or all of these uninfected plants are uninfectable, or immune, and whether degrees of disease development represent intrinsic resistance or a more or less accidental retardation in disease development. In an effort to answer these questions, plants showing apparent degrees of disease development were set in the greenhouse for further observation. Most of these plants developed disease and soon died. Thus the tentative conclusion was reached that degree of infection in plants from susceptible varieties, even after two inoculations, is not a reliable index of degree of resistance in those plants, at least, not of a sufficiently high degree of resistance to be of practical importance. In fact, occasional plants among those showing no infection at all have developed disease and died as though very susceptible. These results may indicate that resistance, at least a low degree of resistance, is not stable in growing plants. Thus one of the first and most essential steps in the study of resistance in individual plants consists in the determination of the stability of resistance with reference to the age and environment of the plant, or the description of such degrees of resistance as may be distinguished.

A simple procedure in the determination of the stability of high resistance in plants with reference to age consists in the repeated inoculation of such plants as fail to be infected by the routine method used in the comparison of plants. A considerable number of plants found uninfected in the fall have been reinoculated and set in the ground for growth the following year. In spite of winter protection, such transplanted roots have been killed outright or so badly damaged that no significant results have been obtained. Inoculated populations left in the ground all winter with some protection have been dug in the spring, and the uninfected plants have been reset in the ground. These have usually failed to grow vigorously after such transplanting; consequently, the reset plants can hardly be regarded as representing the condition of plants that have grown with undisturbed roots. A more promising method of obtaining the desired end consists in the use of cuttings. In the autumn of 1930, cuttings were made from a number of uninfected plants and rooted in the greenhouse. The vigorous plants from these cuttings were inoculated by inserting bits of diseased tissue in the roots and were set in the field in the following spring. A part of the result of this inoculation is given in table 3. Some of the groups of genetically identical plants obtained from cuttings remained free from infection and some groups were infected in part or in all of the individuals. Further trial will be made of these uninfected populations to determine whether infection can be obtained in them; but at present it appears that even from varieties in which 95 percent of the plants are infected in a routine test individuals may be found which are immune to wilt under these summer field trial conditions.

TABLE 3.—*Infection of plants from cuttings the parent plants of which had withstood two inoculations without infection*

Variety	Plant no	Diseased	Healthy
		Number	*Number*
Turkistan	480-1	3	
	480-2		7
	480-6		4
	480-7	4	
	480-9		5
	480-10	2	2
	2-5		11
	2-7	4	3
	1356-5	2	5
Grimm	998-1	4	
Utah Common	1210-1	1	1
	1158-3	4	1
	1158-4	2	1
Montana Common	1300-1		4
	1300-3		2
Kansas Common	1303-2		3
	1303-3		3
	1304-1	4	3
	1304-2	1	2
	1304-4	1	4
	1304-5		2
	1304-7	5	
	1304-8	2	2

Infection that occurred in this trial was usually slight. No symptoms were observed in the foliage. The disease had progressed very slowly after entering the vascular system. Although the fate of these infected plants in the following year was not determined, it seems probable that in most cases the disease would have been outgrown. On the basis of this opinion, such plants may be placed in a highly resistant class in which infection is possible but in which it tends to remain localized.

A tentative third class of plants, having resistance slightly lower than the preceding yet perhaps enough to be of practical importance if possessed uniformly in a variety, is illustrated by the following example: Three plants of Grimm, two without infection, the third with very slight infection, were selected at the end of the inoculation trial in 1930. Cuttings from these plants were grown in the greenhouse and three from each plant were inoculated in the spring of 1931. At the end of the summer none of these plants showed disease, and they remained in the field through 1932, still vigorous and without evidence of disease in the foliage. When they were dug in the winter, they were found badly infected, invasion extending to the center of the root, and they soon died. Degree of infection and behavior differed little in the nine plants. They were much less injured by wilt in 2 years than most Grimm plants in a single summer; therefore, they may be regarded as having a degree of resistance somewhat lower than that described in the previous class.

Thus, by means of these field trials of cuttings from individuals, resistant plants may be classified provisionally as immune, highly resistant, and resistant. Such classification is admittedly very crude and unsatisfactory. It may serve to indicate that resistance appears to exist in varying degrees in plants. In rare individuals immunity may be attained, but comparatively high and low degrees of resistance may be distinguished.

THE NATURE OF RESISTANCE

During the foregoing comparison of varieties with respect to resistance, detailed study has been attempted of the behavior of infection in plants having different degrees of resistance, with a view to describing the differing host-parasite relationship which finds conspicuous expression in these degrees of resistance. In this work methods of approach have been developed and tentative conclusions have been reached which need further confirmation. A discussion of this incomplete work seems warranted, since Peltier and Schroeder (*6*) in a recent publication have reached conclusions radically different from those of the writer.

In the course of the comparative testing of alfalfa seed lots many inoculated plants of resistant or susceptible strains in various stages of disease have been examined and compared. Material has been fixed and stained after being embedded in paraffin, but more frequently living roots have been sectioned with the razor or the sliding microtome. By the latter method discolored areas indicating infection can be examined at the exact point of interest more rapidly and effectively than is possible after the material has been embedded. Sections from living material are fixed in alcohol to hold the bacteria in place and are stained in the usual manner. Thus the relation of the bacteria to large areas of the host tissue may be surveyed in a relatively short time.

Attention was first directed to the initial step in infection, namely, the development of the bacteria in parenchymatous tissue around the wound to which the bacteria were applied. Here it was found that in plants with no vascular invasion at the end of a routine test for resistance, there is usually no invasion of parenchymatous tissue or but a very slight invasion which has not reached the vessels. In plants in which vascular invasion is slight, invasion at the point of inoculation is slight and is not found elsewhere along the invaded vessels. In very susceptible plants invasion of parenchymatous tissue at the point of inoculation is relatively rapid and abundant, and vascular invasion is soon followed by parenchymatous invasion at more or less widely scattered points along the invaded vessels. Occasionally very conspicuous exceptions occur in which extensive invasion of parenchymatous tissue at the point of inoculation is not followed by extensive vascular invasion, but these exceptions appear to form a very small distinct class which need not confuse the discussion here.[4]

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[4] In the exceptional plants referred to, the parenchymatous tissue appears to react to invasion by hypertrophy and even by slight hyperplasia. The region of inoculation develops a gall-like swelling. The bacteria usually pass to the very center of the root along the enlarged wood ray cells. Few vessels are invaded, and these may be scattered to the center of the root. Such plants have been found thus far only in strains of Turkistan alfalfa.

The foregoing results indicate that resistance first finds expression in the failure of the bacteria to establish themselves rapidly and extensively in the parenchymatous tissue of the host, either at the portal of entry, or later from the invaded vessels.

Following the examination of infection in populations presumably more or less heterogeneous, similar comparisons were made with plants of known uniformity, that is, with cuttings from previously tested plants. The supply of such plants has been small, both in quantity and in diversity of origin; therefore, methods were devised whereby it could be used most economically. Chief among these were the inoculation of pieces of roots in an incubator and the reciprocal grafting of plants. The results of the preliminary tests are as follows.

Pieces of roots taken from large plants in the fall were stored under controlled environmental conditions, and infection has been obtained in such pieces as well as in entire plants. The procedure has been used both to test rate of penetration of the bacteria in susceptible plants under different environmental conditions and to compare penetration in susceptible and resistant roots. Results obtained in this way support observations made in growing plants. Very little penetration of the bacteria into the phloem of roots of well-attested resistance has occurred, while abundant penetration of bacteria into roots of susceptible plants has been obtained.

The most spectacular contrast in the development of the bacteria in resistant and susceptible plant tissue has come from inoculation of grafted plants, though this work is as yet meager in quantity. The grafting is easily accomplished between roots of the same diameter, both in the fall with plants from the field and in the spring with greenhouse seedlings ready to set in the field. The usual whip graft has been used, the union tightly bound with twine and covered with melted beeswax. The grafts are packed in moist sphagnum, with a little of the crown exposed, and maintained at a temperature of about 25° C. in a moist chamber for 2 or 3 weeks to hasten the union. The plants are then set in soil with protection from drying until vigorous growth starts. Inoculation has occasionally been made on the cut surfaces at the time of grafting, but usually in the susceptible portion of the graft at the time of planting.

The two portions of the graft appear to maintain essentially their original susceptible or resistant characteristics in this union, although a susceptible top on a resistant root lives longer after infection than it does on its own decaying root. Through the use of grafted plants bacteria can be introduced directly into unwounded and continuously functioning vessels of resistant tissue without having to pass through parenchymatous tissue in which, as previous observation indicated, they were hardly able to penetrate.

A typical contrast of bacterial development in tissues of immune root inoculated in this way with that in a susceptible root is furnished by a fortunate graft in which about 4 cm of immune Turkistan root was inserted in the taproot of a highly susceptible nonhardy plant. This graft was made in December 1931. The lower portion of the susceptible root was inoculated, and the plant grew slowly in the greenhouse during the winter. In the spring it was set in the field, where it grew vigorously, showing no symptoms of disease. When dug in October the taproot was about 2 cm in diameter and the graft unions were smooth, though visible. The crown and taproot were then split vertically; one half was waxed over the wound and set in the greenhouse, and the other half was prepared for microscopic examination. On December 19 the foliage of the greenhouse plant showed symptoms of wilt and was then examined. Sections prepared at the earlier and later dates from this plant show differences only in degree of disease. The entire root, including the inserted portion, was deeply discolored. The new growth formed since the union was a wide band each of summer and autumn wood sharply distinguished. The lower susceptible portion of the root was severely diseased, with extensive invasion of the fall wood both in vessels and in parenchymatous tissue. The upper susceptible portion was less severely invaded, although bacteria had advanced to some of the outermost vessels. In the immune insert, which was almost as deeply discolored as the rest, the bacteria were found only in a few vessels of the summer wood and not anywhere in parenchymatous tissue. Extensive gum formation occurred in the susceptible portion, but only in the summer wood of the immune insert. The discoloration of the immune portion was due largely to the soluble stain, which may have been produced in the susceptible part. So far as could be discovered, vascular connection through the inserted portion was open, permitting distribution of bacteria through it from the infested vessels at either end, and perhaps the bacteria found there had arrived by this route. Previous trials with ink in grafts had demonstrated open vessels through the unions permitting ink particles to pass, though many vessels had sharp turns conducive to clogging.

In other inoculated grafts of resistant tops on susceptible roots, and vice versa, made at about the same time, similar results have been obtained. Disease has progressed in the usual manner in the susceptible portion. Discoloration extends far into the resistant part of the union, although few vessels in that resistant part show clear evidence of bacterial development in those vessels by the presence of matlike colonies over pits.[*] Thus it appears that immunity and very high resistance are manifest not only by failure of the bacteria to develop in the intercellular spaces of the parenchyma of an inoculated plant, but also by a much less luxuriant growth in the pits of those vessels. In no case have bacteria been found invading parenchymatous tissue in the highly resistant part of grafts, however extensively this has occurred in the susceptible part.

From the writer's previous studies of this disease the conclusion has been drawn that without invasion of parenchymatous tissue vascular invasion does not proceed far; and thus if this apparent resistance of the parenchymatous tissue to invasion continues to be found in all plants which are not infected or but slightly infected by repeated inoculation in the usual routine, the resistant character seems to be manifest chiefly in the parenchymatous phase of invasion. The nature of this resistant character is not apparent. The gathering, testing, and increasing of resistant plant material for comparison is in progress, and a more comprehensive comparison of such material is planned.

A theory of the origin of resistance very different from that just outlined has been stated recently by Peltier and Schroeder (*6, p. 2*).

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* * * The usual or normal progress of the bacteria from their entry until the death of the plant ensues has been followed.

> In the main it has been found that resistance in some alfalfas is associated with certain morphological features, particularly in the root, which inhibit rapid development and invasion of the vital tissues by the bacteria. These morphological differences in susceptible and resistant sorts are inherent, though not absolute, since any variety or strain of alfalfa is made up of a widely diverse lot of individuals. It is for this reason that not a single variety or strain of alfalfa has been found which is completely resistant.
>
> * * * Thus while resistance in alfalfas to wilt is associated with root structure, it is also true that inhibiting or accelerating the rate of growth of either susceptible or resistant sorts will modify the root structure to such an extent that susceptible sorts will become more resistant or resistant alfalfas more susceptible.
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> * * * There appears to be no direct evidence in any of our physiological or microchemical studies to show that any internal physiologic function of the plant makes one variety more resistant than another, except insofar as morphological modifications may occur under different environmental conditions.

The morphological characteristics of resistant varieties are stated to be as follows: (1) Vessels of smaller diameter, more angular, with heavier wall thickenings; (2) vessel segments shorter, with more obstructions from remaining vestiges of the septa; (3) vessels arranged in groups, with less contact and more intervening fibers; and (4) less frequent contact of vessels with parenchymatous cells. Peltier and Schroeder illustrate these differences by contrasting photomicrographs, but give no measurements of length or diameter of vessel segments. Measurements made by the writer from their photomicrographs (*6, pl. 7*) showing difference in diameter of vessels in a plant of a resistant and a susceptible variety show that the average outside diameter of vessels in the resistant root is about 23μ and in the susceptible root about 38μ. The writer has examined sections of many resistant and susceptible plants and has not observed such contrast in vessel diameter. However, in such comparisons, measurements are a safer guide than observation, and therefore, in July 1933, plants of 3 resistant and 4 susceptible varieties under test in the field were sectioned for examination. Median longitudinal sections were stained lightly with thionine, and the length and diameter of vessel segments of at least 10 representative vessels distributed across the diameter of the root were measured. The average of these measurements was found, and finally the average of such measurements from 5 plants. The results are given in table 4. No significant difference appears in either length or diameter of vessel segments between the resistant and the susceptible varieties. In all plants vessel diameter is approximately the same as that shown in the cited illustrations of a susceptible plant (*6, pl. 7, b*).

TABLE 4.—*Comparison of average length and diameter of vessel segments in roots of resistant and susceptible varieties of alfalfa under test for resistance to wilt*

[Measurements made in plants taken from the field, July 19, 1933]

Resistant variety	Average length	Average diameter	Susceptible variety	Average length	Average diameter
	μ	μ		μ	μ
Turkistan	104	32	Grimm	84	38
Hardistan	90	38	Cossack	80	38
Ladak	86	36	Canadian Variegated	88	36
			South Dakota Common	80	38

The writer has sought among the plants in his selections for evidence of the remaining differences between resistant and susceptible plants described by Peltier and Schroeder and has failed to find any of them. It will be very interesting indeed if these investigators have found an environmental condition in which resistant and susceptible varieties show different internal structures, but such structural differences can hardly be regarded as a cause of resistance when similar contrasts in resistance are shown by the same varieties grown in an environment in which these structural differences do not occur. The structural differences between roots of resistant and susceptible varieties here described appear closely similar to those observed by the writer in plants responding to a short period of illumination with short internodes and with long internodes, respectively, when both are grown in short days.

It should be pointed out that Peltier and Schroeder appear to the writer to have built their morphological theory of resistance upon a conception of the development of the disease in the plant which differs in one important respect from that held by the writer (*2*, *4*). The difference in opinion concerns the route by which the bacteria spread within the plant, and may be stated thus. Both agree that in entering a plant through a wound the bacteria first establish themselves in parenchymatous tissue, whence they pass into vessels. From this point interpretations differ. The writer finds parenchymatous invasion arising along those vessels first invaded and believes that by passing between cells of this tissue the bacteria invade other vessels in the same manner in which the first vessels were penetrated at infection. Peltier and Schroeder do not describe such parenchymatous invasion, progressively increasing from initial infection, but describe the passage of bacteria from vessel to vessel by penetration of the thin wall separating opposed pits of contiguous vessels. Although the writer has recognized that passage of bacteria from vessel to vessel does occasionally take place by the dissolution of cell walls (*4*, *p. 70*, *fig. 18*), he has been unable to find convincing evidence that passage occurs through pores in pits as described by Peltier and Schroeder (*6*, *pp. 7–8*). In fact, the writer is unable to verify the existence of the pores described in the cell membrane. The photomicrographs cited as showing these pores or reticulated condition of the membrane (*6*, *pl. 1*, *B*, *C.*) appear to show only a refractive effect of the lignified thickening of the wall, not pores in which bacteria can or do find lodgment and development. In a recent paper, Bailey (*1*) gives illustrations and a description of vestured pits that appear to identify as vestured pits the pit condition in alfalfa illustrated by Peltier and Schroeder.

It appears probable to the writer that Peltier and Schroeder overlooked the presence of parenchymatous invasion in the early stages of disease development, widely scattered and tenuous as it often is along the invaded vessels. For instance, they state (*6*, *p. 27*):

> As a rule plants in which bacteria are present in the parenchymatous tissues in the fall do not survive the winter or spring because of the small amount of root reserves found in plants in this stage of disease development.

This statement does not accord with the writer's experience. In the spring of 1933 special search was made for local invasions of parenchyma in plants overwintering with but a trace of disease. Among plants brought from an old field, invasions in parenchyma were demonstrated by staining in 80 percent of the plants examined, and in 90 percent of overwintering plants artificially inoculated the previous summer. Invasion of parenchyma has been found, in the writer's opinion, sufficiently associated with all stages of active disease development to account for progress of bacteria to new vessels.

RESISTANCE AS INFLUENCED BY SELECTION

A single experiment indicating the increase in resistance that may be obtained from selection alone will be reported here. In the summer of 1931 a small field planting of cuttings of highly resistant plants, well separated from any susceptible plants, set seed. The planting consisted largely of selections from susceptible common alfalfa, with a few plants of Ladak and of Turkistan origin. The open-pollinated seed from each individual plant represented was kept separate and plants from this seed were tested for resistance the following year, in some cases in comparison with a part of the original seed lots. The results of this test are summarized in table 5. In the 1930 test of the seed lots the plants were inoculated twice; in the 1932 retrial of those lots and in the test of the seed from the selections the plants were inoculated but once. The percentage of resistance given for the selections is an average of several trials in the same plot, the number of plants included ranging from 60 to 370. Differences between resistance in parent stock and selections in the first three instances and in Ladak do not, in the writer's opinion, represent a significant increase in resistance in the selection; but in all other cases the increase is regarded as highly significant. This result indicates that considerable increase in resistance can be obtained from some of the resistant plants selected by the procedure here described. Moreover, the resistant selections in this test appeared to have the general character of the stock from which they came and were not of the early-dormant Turkistan type. Additional selections from this plant material and from other sources are being made for a further test of the possibility of increasing resistance in strains from selected plants.

TABLE 5.—*Comparison of resistance in some highly susceptible seed lots with resistance in the F_1 population of selected resistant plants from those seed lots*

[Resistance is represented by the percentage of plants remaining uninfected at the end of a routine inoculation trial]

Parent stock no.	Variety	Resistance of parent stock		Resistance in F_1 population from numbered selected plants (1932) [b] [c]				
		1930 [a]	1932 [b]	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1210	Utah Common	0.5		11.7	8.7			
1225	Kansas Common	.5	14	15				
1245	Utah Common	1	22	22				
1289	Arizona Common	1.4	3.4	23.5	16			
1300	Montana Common	1.4	3.4	25	16			
1301	Kansas Common	2.4		40	23.3			
1302	Do	1.8	7	49				
1303	Do	32.4		35.3	38.7	37.5	66.6	
1304	Do	2.8	7	26.6	33.1	31	29.8	40
1334	Ladak	.30		36				
1335	Grimm	1		31				
1356	Turkistan	24		66	43.7	59.3		

[a] Inoculated twice. [b] Inoculated once [c] Average of several trials.

SUMMARY

Alfalfa plants grown from seed from various sources have been inoculated at Madison, Wis., during 3 years for two purposes, namely, the comparison of resistance in the sources represented and the selection of resistant plants from which resistant strains may be developed.

Several procedures by which resistance in plants from different seed lots may be compared are described and evaluated.

Alfalfa from Turkistan and Ladak shows far more resistance than that from any other source from which seed has been tested thus far. However, occasional highly resistant plants are found from other sources.

On the basis of the routine inoculation used in the tests, resistant plants have been tentatively grouped in three classes, namely, immune, highly resistant, and resistant.

Resistance appears to be exhibited largely in the parenchymatous tissue of the plant through which the bacteria make little or no progress in resistant plants, and somewhat through the less rapid multiplication of the bacteria in the vessels.

No evidence has been found of morphological differences distinguishing resistant plants.

Increase in resistance in open-pollinated progeny from some of the selected plants has been obtained.

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A GALL SIMILAR TO CROWN GALL, PRODUCED ON GYPSOPHILA BY A NEW BACTERIUM [1]

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INTRODUCTION

Galls on the crown and roots of *Gypsophila paniculata* L. were first brought to the attention of pathologists of the Bureau of Plant Industry in the summer of 1932, when a grower submitted specimens of galls that had occurred in an ornamental-plant nursery in the eastern part of the United States. Galls on *Gypsophila* apparently were seen for the first time in 1931 by eastern growers of this important ornamental plant. However, neither the damage to plants nor the financial loss was extensive; consequently the growers did not at that time bring the disease to the attention of Federal workers interested in plant-disease problems.

In 1932, of 12 nurserymen who produced *Gypsophila paniculata* for the wholesale trade, only 2 were familiar with the disease, and one of these minimized his losses. He admitted that he understood the potential danger but stated that his loss had been less than 1 percent. The losses of the nurseryman who submitted the galled plants in 1932 amounted to about 25 percent.

A note regarding the discovery of the disease was published in the autumn of 1932.[2]

THE DISEASE

The galls occur principally on grafted plants in the region of the graft. They are of a soft nodular type, ½ to 3 cm in diameter (fig. 1, *A*), and may extend around the greater part of the stem or root, eventually causing the death of the plant. It is the practice of the eastern *Gypsophila* growers to lift seedling plants in the fall to use later for grafting with the desired variety. Should their field plants be galled in the summer, they are worthless when dug.

Crown gall of ornamental plants, of vegetables, and of fruit trees, which is produced by *Bacterium tumefaciens* Smith and Town., is so well known and wide-spread that no surprise is manifested when a new host plant for the disease is found. Consequently, when the *Gypsophila* gall was received and examined and its outward appearance was observed to be very like crown gall, it was at first thought that *Gypsophila* was a new host for crown gall and that the disease was not new.

The routine work for identification, however, changed this idea. Cross sections examined under the microscope showed water-soaked areas and masses of bacteria streaming from the tissues, characters that are unlike crown gall. No nematodes or fungi were present. The disease was not crown gall, but there was a possibility that it might be related to one of the other bacterial plant tumors, namely,

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[1] Received for publication Mar. 19, 1934, issued July 1934

[2] BROWN, N. A. ANOTHER GALL-FORMING BACTERIUM. Phytopathology 22: 924-925, illus 1932.

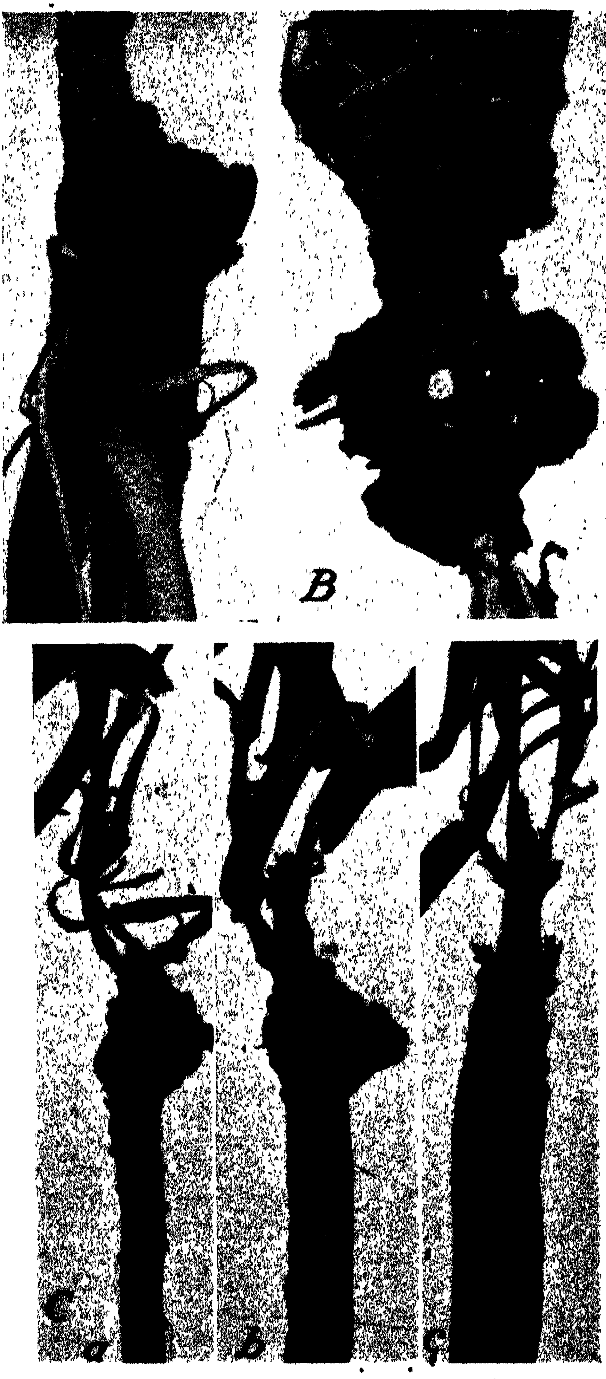

FIGURE 1.—*A*, *Gypsophila paniculata* gall from an Eastern State. *B*, *G. paniculata* gall 5 weeks old, produced by inoculating with an organism isolated from *A*. *C*, Seedling *G. paniculata* plants: *a* and *b*, inoculated with 2 different colonies reisolated from gall *B*, which produced these galls in less than 1 month; *c*, control plant. Natural size.

the pocket disease of the sugar beet, produced by *Bacterium beticola* (Smith, Brown, and Townsend) Potebnia; the olive knot, produced by *Bact. savastanoi* E. F. Smith; the oleander gall, produced by *Bact. savastanoi* var. *nerii* C. O. Smith; or a canker of ash trees, produced by *Bact. savastanoi* var. *fraxini* Brown.

A further study of the structure of the *Gypsophila* gall, however, showed that it had features unlike these last-named tumors and could not be classed with them. There were no gum pockets in any of the *Gypsophila* galls received for examination. There were a few brown areas in some of them, but whether the discoloration was the beginning of the break-down of the gall tissue due to the invading organism or to a reaction of the gall tissue to the byproducts of this organism is not known. No browning occurred in the galls produced by inoculation. The *Gypsophila* galls are so soft that disintegration occurs very easily.

The galls were studied in cross section (fig. 2) and in the water-soaked areas motile bacteria were seen in the cells. Some of the cells were filled with them, others were partly filled, while many had none. A few of the tumor cells were packed with crystals. The cells near the periphery of the galls contained the greatest number of bacteria. The causal organism of crown gall has not been seen in the natural gall.

The structure of the *Gypsophila* galls was found to be much like that of crown gall. Nests of rapidly developing cells could be distinguished in which parenchyma and sclerenchyma cells were mixed irregularly (figs. 2, *A*; 3, *C*).

ISOLATIONS AND INOCULATIONS

COLONIES

An organism was isolated from several galls, the same type of colony appearing on the plates poured from each gall. The colonies were abundant and apparently consisted of the pathogene responsible for the disease. They appeared in 24 hours, were translucent white in reflected light, circular, 2 to 4 mm in diameter, slightly raised in the center, and finely granular (fig. 2, *B*). Buried colonies were mostly lens-shaped, but some were round. There were no irregular colonies. In 3 days they were a creamy yellow, 4 to 7 mm in diameter, and on thinly sown plates, 8 to 11 mm. Both rough and smooth colonies appeared on the plates isolated from the same gall, but the smooth colonies predominated. Both types of colonies were used for inoculations and produced galls of similar size, although the rough type produced them a little more slowly than did the smooth type.

By means of needle pricks grafted *Gypsophila* plants were inoculated at the crown and also on the stems with several of the isolated colonies. There was a beginning of gall formation at the crown in 7 to 9 days. In 2 weeks light-colored nodular galls 1 to 1½ cm in diameter showed at the crowns. Some of the inoculated plants were slow in showing infection, but in 3 weeks all had definite galls (fig. 1, *B*). When they were 2½ to 3 cm across, quite frequently disintegration began (fig. 4, *A*). Cankers, instead of galls, were produced on the inoculated aerial stems. On the 24 plants inoculated, the infection on crown and stem was 100 percent.

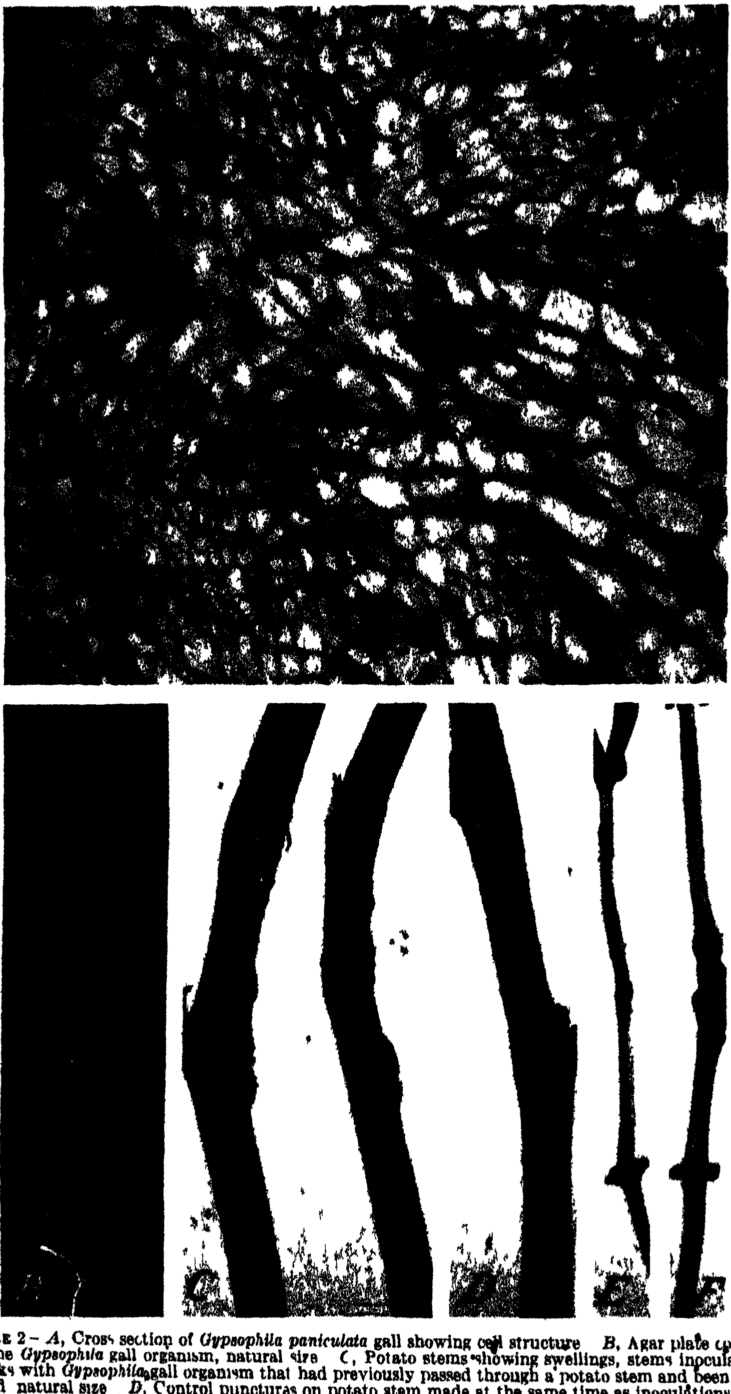

FIGURE 2.—*A*, Cross section of *Gypsophila paniculata* gall showing cell structure. *B*, Agar plate colonies of the *Gypsophila* gall organism, natural size. *C*, Potato stems showing swellings, stems inoculated 3 weeks with *Gypsophila* gall organism that had previously passed through a potato stem and been reisolated; natural size. *D*, Control punctures on potato stem made at the same time as inoculations in *C*; natural size. *E*, *Saponaria vaccaria* inoculated with *Gypsophila* gall organism 4 days. *F*, *S. vaccaria* inoculated with *Gypsophila* gall organism 11 days. Organism is most active on this host, which is a relative of *Gypsophila*. *E* and *F* slightly reduced in size.

Roots of seedling *Gypsophila* plants inoculated at the crown likewise gave 100 percent infections. The galls formed about as rapidly as on the older grafted plants (fig. 4, *C, a*). Hot moist conditions favored gall development. Platings were made from the galls produced by inoculation, and the organism was recovered. *Gypsophila* plants inoculated with this reisolated strain developed galls of the same type as rapidly as did plants inoculated with the original strain (fig. 1, *C, a, b, c*).

At the time the *Gypsophila* plants were inoculated, stems of tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.), carnations (*Dianthus caryophyllus* L.), garden balsam (*Impatiens balsamina* L.), sugar beets (*Beta vulgaris* L.), Paris daisies (*Chrysanthemum frutescens* L.), nasturtiums (*Tropaeolum* L.), and other plants were inoculated with the same organism. No galls formed on any of them, but well-defined swellings occurred on the potato stems. Isolations were made from the potato swellings, the organism was recovered and outgrowths similar to the natural galls were obtained by inoculating *Gypsophila* plants with this potato isolation (fig. 3, *A*). Potato stems, inoculated with the potato isolation, developed swellings like those caused by the original organism, but neither galls nor cankers (fig. 2, *C, D*). Inoculations made into potato tubers attached to the plant at different stages of growth produced no outgrowths.

The common hosts of *Bacterium tumefaciens*, such as Paris daisy, sugar beet, *Ricinus* L., geranium (*Geranium* L.), and garden balsam, did not prove susceptible to the *Gypsophila* gall organism when they were inoculated with it, nor did *Bact. tumefaciens* produce any trace of outgrowth on roots or stems of *Gypsophila paniculata* (fig. 4, *C, b, c*). The roots and stems of *G. paniculata* were also inoculated with the olive-knot organism (*Bact. savastanoi*) and the ash-canker organism (*Bact. savastanoi* var. *fraxini*) with negative results.

FILTRATES AND PLEOMORPHIC FORMS

The juice of crushed *Gypsophila* galls was passed through Chamberland L 3 filters, and *Gypsophila paniculata* and *Lychnis chalcedonica* L. plants were inoculated with the filtrate. No galls resulted. Filtrates from beef-bouillon cultures were also used for inoculations, with the same result. A portion of the filtrates was held in sterile tubes for several weeks, then cultured repeatedly on hardened agar plates, according to the technic of Hauduroy [3] and of Hadley.[4] With this procedure the filtrates passed through the granular and coccus stages and later reached the normal rod form again, but when the cultures arrived at the rod form the ability to infect the *Gypsophila* plants was lacking. The cultures would not produce galls.

The writer had tried out the method previously with three different strains of *Bacterium tumefaciens*, namely, the hop, daisy, and peach strains. Neither the crushed-gall filtrates nor the beef-bouillon-culture filtrates of the three strains produced galls when inoculated into susceptible plants. Portions of the sterile filtrates were held in tubes for a few weeks to a few months, then cultured for some time on hardened agar plates. From the granules, the coccus form developed, and later from the coccus the normal rods. Inoculations were made into young

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[3] HAUDUROY, P LES ULTRAVIRUS ET LES FORMES FILTRANTES DES MICROBES 292 pp Paris 1929

[4] HADLEY, P, DELVES, E, and KLIMEK, J THE FILTRABLE FORMS OF BACTERIA I A FILTRABLE STAGE IN THE LIFE HISTORY OF THE SHIGA DYSENTERY BACILLUS Jour Infect Diseases 48 1-159 1931

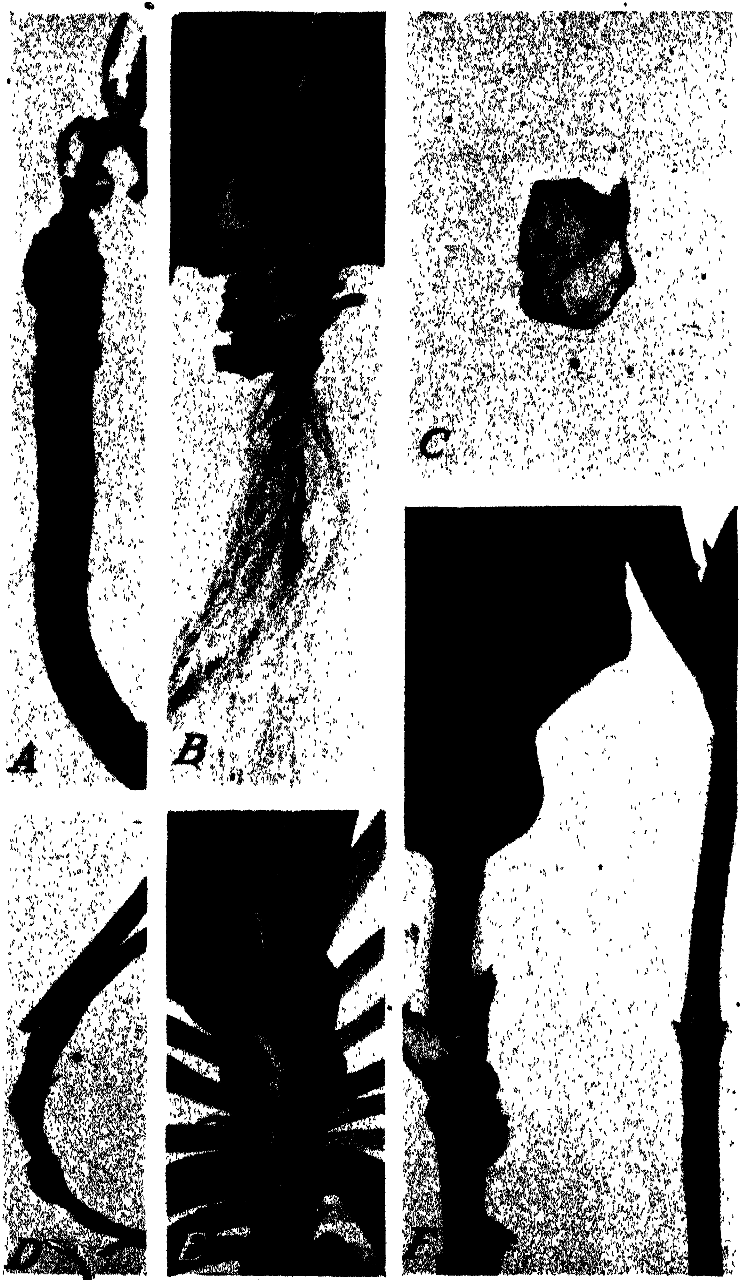

FIGURE 3.—*A*, Gall on seedling *Gypsophila paniculata*, produced by inoculation with a strain of the organism reisolated from a potato stem on which a swelling was produced but not a gall. The potato reisolation, however, produced galls on *Gypsophila* root. Photographed 5 weeks after inoculation. *B*, Gall on *Silene armeria* produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *C*, *Gypsophila* gall 1 month old cut across to show internal structure. *D*, Gall on *Dianthus plumarius* (garden pink) produced by inoculation with the *Gypsophila* gall organism; time, 2 months. *E*, *Dianthus barbatus* (sweet-william) inoculated with *Gypsophila* gall organism 2 months; no infection. *F*, At left gall on *Lychnis chalcedonica* produced in less than 1 month by inoculation with the *Gypsophila* gall organism; at right, control punctures on *L. chalcedonica*. All natural size.

susceptible plants with both coccus and rod forms, but no trace of crown gall resulted.

With the *Gypsophila* gall filtrates the transition stage from coccus to rod came more quickly than with the filtrates of *Bacterium tumefaciens*, and it was hoped pathogenicity accompanied this less tedious manipulation. It did not prove to be the case, however, as no infection followed inoculations into susceptible plants.

THE CAUSAL ORGANISM

CULTURAL CHARACTERS

BEEF-INFUSION AGAR PLATES.—White colonies are visible in 24 hours after pouring plates from macerated gall tissue incubated at 22° to 25° C. In 48 hours they are deep cream to wax yellow and range from 2 to 4 mm in diameter; they are smooth, circular with entire margin, shining, convex, a little thicker in the center. In 4 days the colonies on thinly sown plates are 4 to 11 mm across, and in some there is a margin. At 6 days the color is mustard yellow; later, primuline yellow.[5]

After the organism has been cultured a few weeks in artificial media, plates poured from a 1-day beef-bouillon culture may show more rough than smooth colonies. These are much the same as the colonies that appear from the isolation material. Occasionally there is a rough colony that has a high convoluted surface. The color of week-old cultures is primuline yellow.

BEEF-INFUSION AGAR SLANTS.—There is a thin spreading growth, usually papillate but sometimes smooth, on beef agar slants in 24 hours. Under the hand lens it has a metallic luster on beef agar. The pH is 6.8 at temperatures of 25° to 30° C. At 4 days growth is abundant, butyrous, translucent; at 7 days the metallic luster has disappeared and there are many crystals. The color of the growth is Naples yellow.

BEEF-INFUSION BOUILLON.—Clouding is prompt, being quite definite in 7 hours at 34° C.; at 30° there is good clouding in 24 hours; in 48 hours a yellow pellicle has formed which falls readily.

THAXTER'S POTATO-DEXTROSE AGAR SLANTS.—The growth is spreading but not so rapid as on beef agar; it is rough, butyrous, cream-colored, and continues so when a week old.

POTATO CYLINDERS.—There is a thin cream-yellow growth in 1 day; it is still scanty at 6 days, with the color buff-yellow, and the potato is slightly discolored. After 30 days the color is Naples yellow, but the medium has not darkened further.

COHN'S SOLUTION.—Growth is rapid and heavy in Cohn's solution, and a complete pellicle forms with larger irregular crystals hanging from it. At first the pellicle is white but changes in 7 days to Naples yellow. The medium becomes cream-colored and has a yellow precipitate.

USCHINSKY'S SOLUTION.—Growth is prompt in this medium, and there is a heavy white pellicle in 2 days; in 12 days the pellicle is cream-colored and the medium Naples yellow.

FERMI'S SOLUTION.—Growth occurs readily but is not so heavy as in Uschinsky's solution. There is a white pellicle in 2 days, which changes to mustard yellow in 12 days.

PHYSIOLOGIC CHARACTERS

LIQUEFACTION OF GELATIN.—No liquefaction begins in beef-gelatin stabs until the cultures are a month old. The liquefaction continues slowly and is not completed until after 4 months. The cultures were kept at 14° to 15° C. and three different lots of beef gelatin, having pH 6.5, 7.0, and 7.3, respectively, were used.

Colonies on beef-gelatin plates are cream-colored until 6 days old, when they become mustard yellow. Both smooth and rough colonies occur on all plates, with the rough type more abundant; they are translucent in reflected light, transparent in transmitted light. In 3 weeks there are little hollows around the colonies, and in 4 weeks liquefaction is definitely visible but continues very slowly. In heavily seeded plates the gelatin is entirely liquefied in 4 months; in sparsely seeded plates the liquefaction extends 1 cm beyond the colony. Feathery branched crystals are formed on the plates and in the stab cultures.

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[5] The color readings in this paper are based on the colors in the following publication. RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE 43 pp., illus. Washington, D.C. 1912.

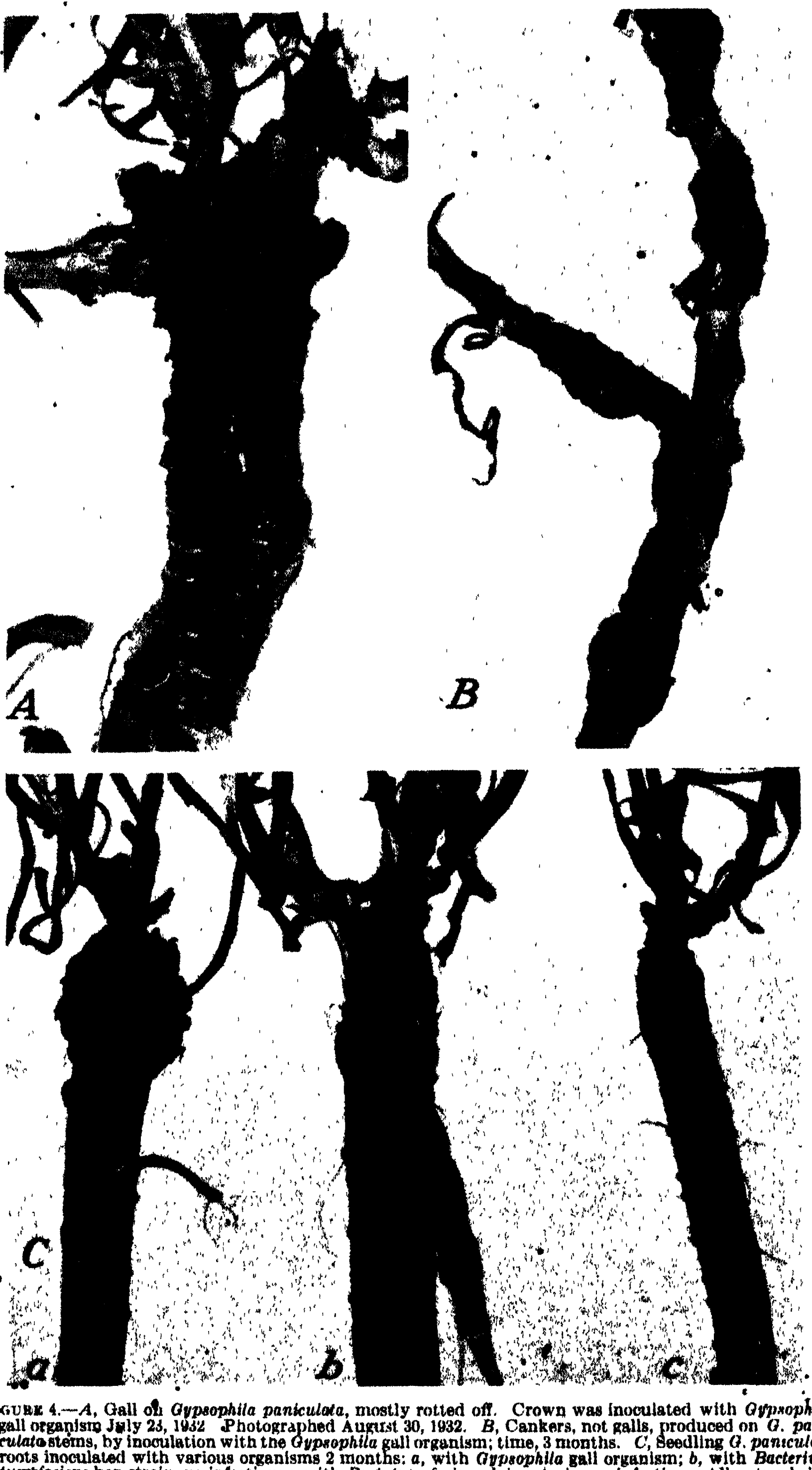

FIGURE 4.—*A*, Gall on *Gypsophila paniculata*, mostly rotted off. Crown was inoculated with *Gypsophila* gall organism July 23, 1932. Photographed August 30, 1932. *B*, Cankers, not galls, produced on *G. paniculata* stems, by inoculation with the *Gypsophila* gall organism; time, 3 months. *C*, Seedling *G. paniculata* roots inoculated with various organisms 2 months: *a*, with *Gypsophila* gall organism; *b*, with *Bacterium tumefaciens* hop strain, no infection; *c*, with *Bact. tumefaciens* daisy strain, no infection. All natural size.

Milk.—Coagulation of milk occurs at 9 days, with very little whey. A light straw-colored pellicle is visible at 6 days. The curd digests slowly, beginning at 17 days and being completed in 90 days, at which time the color of the milk is tawny.

Blood serum.—A very good growth takes place on blood serum, but there is no liquefaction. The mustard-yellow color at 2 days becomes the deeper primuline yellow at 12 days. The blood serum grays a little at the base. Cultures 2 months old show no trace of liquefaction.

Reduction of litmus.—There is a dull pink color throughout litmus-milk cultures in 24 hours, and the original color, pale aniline lilac, is changed to pale lobelia violet; in 48 hours this color is still duller except at the surface of the liquid. In general the color change in comparison with the uninoculated tubes is slight. In 8 days the litmus has faded to lavender-gray and in 10 days it is reduced, the color now being tilleul-buff; there is a yellow pellicle and yellow precipitate of the growing organism. Coagulation usually takes place on the ninth day, sometimes on the eighth day, after litmus milk has been inoculated. Digestion of the curd is slow, not being completed before 3 months. The medium at this time is dull reddish purple, and the organism is still alive.

Reduction of nitrates.—Nitrates are reduced to nitrites. Tests were made on nitrate-bouillon cultures 3 and 5 days old, with the sulphanilic acid α-naphthylamine test. There was a good red color in all the tubes, indicating the presence of nitrites. Other cultures when 25 and 30 days old were tested with the same result.

Indol production.—No indol is produced. Tests were made on the organism growing in tryptophane broth at 3, 5, and 30 days, respectively, with the Ehrlich-Bohme method. *Bacillus coli* (Escherich) Migula, which produces indol, grown as a control and tested at the same time, gave a good pink color, a positive test. The *Gypsophila* gall organism showed no pink color.

Fermentation of carbohydrates.—The organism is not a gas former. It was tested in fermentation tubes in the presence of the following carbon compounds. Dextrose, saccharose, lactose, glycerin, and mannite. A 1-percent solution of each was made in a 1-percent water solution of Difco peptone. Besides heavy growth in the open arm of each tube there was growth in the closed arm of the tube with each compound except glycerin. No gas was produced. Acid was produced in all the solutions but that of lactose. A second test was made with the same result. The pH readings were taken just before inoculation with the *Gypsophila* gall organism, also 20 and 27 days after, as shown in table 1.

The same carbohydrates added to synthetic agar with brom cresol purple as indicator, made according to the formula given in the Manual of Methods,[6] were also tested for fermentation. Growth occurred promptly, as did the acid reaction with saccharose, dextrose, glycerin, and mannite, the yellow color change in the purple medium beginning in 18 hours and being complete in 48 hours. There was growth in the lactose cultures but no color change.

Table 1.—*Acid production by the Gypsophila gall organism after 20 and 27 days of growth in 1-percent sugar solutions added to 1-percent Difco peptone*

[Acidity indicated by pH readings]

Number of days of growth	pH of solution containing peptone and indicated carbohydrate					pH of plain peptone water
	Dextrose	Lactose	Saccharose	Mannite	Glycerin	
0	6.5	6.5	6.7	6.7	6.5	6.6
20	4.2	7.0	5.0	5.0	6.4	7.4
27	4.4	6.8	5.0	4.9	6.5	7.3

Diastatic action.—There is no reduction of starch. On starch-agar plates streaked with the organism and tested at 5, 9, 12, and 16 days, respectively, there was no clear zone in the medium.

Ammonia production.—The organism produces ammonia. Tests were made with old and young beef agar, beef bouillon, and peptone-water cultures, using filter paper saturated in Nessler's solution and suspended in the tubes. When the cultures were heated in a water-bath, browning of the paper began immediately. In cultures 2 weeks old the brown color was much more pronounced than in the 3- and 5-day cultures.

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[6] Society of American Bacteriologists, Committee on Bacteriological Technique. Manual of methods for pure culture study of bacteria. 130 pp., illus. Geneva, N.Y. 1930.

Hydrogen sulphide production.—The organism may produce a trace of hydrogen sulphide. Agar, beef-bouillon, and potato-cylinder cultures were tested by suspending lead acetate paper in the culture tubes. When the beef-bouillon cultures were 8 days old there was slight darkening at the tip ends of the paper, indicating hydrogen sulphide production; there was slightly more darkening of the paper when cultures were 2 weeks old. There was no darkening of the paper in the other cultures.

The organism was then grown on lead acetate agar. A heavy yellow growth occurred on the red agar, but there was no dark line or any browning indicating the presence of hydrogen sulphide. A second and third test with the lead acetate agar was made which likewise showed no hydrogen sulphide production.

Toleration of sodium chloride.—The organism grows in pH 6.5 beef-infusion bouillon containing 6, 7, 8, or 9 percent of sodium chloride. There is no growth in beef bouillon containing 10 percent of sodium chloride.

Oxygen relations.—The organism is a facultative anaerobe. In tests made with shake cultures of agar and gelatin, tiny clumplike colonies grew throughout the medium. When long sterile cover glasses were dropped on hardened agar plates streaked with the organism, growth was more abundant at the edges of the cover glass, but it extended inside the edges, showing the anaerobic tendencies. In agar and gelatin stab cultures it grew at once at the bottom of the tube, and in synthetic-dextrose-indicator-agar shake cultures, the purple color of the medium was changed to red at the bottom of the tube as quickly as at the surface of the medium.

Thermal relations.—The organism grows at temperatures ranging from 5° to 40° C. The optimum temperature is about 34°; it does not grow at 0° nor at 42° and only faintly at 5° and 40°.

The thermal death point is between 52° and 53° C., when fresh beef-bouillon cultures, pH 7.0, are exposed in a water bath for 10 minutes.

Growth in beef bouillon.—The best growth in peptone-beef-infusion bouillon takes place at pH 6.5 to 6.7, although the organism has a wide range, extending from pH 5.1 to 9. There is no growth at pH 4.9 or 9.1. At pH 5.1 there is only a faint growth; at pH 9 there is a fair amount of clouding with pellicle.

Effect of desiccation.—The organism is only slightly resistant to drying. Sterile cover glasses smeared with young beef-bouillon cultures and dried at room temperatures (24° to 27° C.) were dead in 5 days.

Effect of freezing.—The organism can withstand freezing temperatures for more than 45 days. Immediately after being transferred, beef-agar and beef-bouillon cultures were placed at temperatures of −21.7° to −23.9° C. Some were removed after 7, 9, 12, and 45 days. All showed typical growth within 1 day after the medium melted.

Longevity.—The organism lives for 7 to 8 months in sterile milk and beef bouillon, pH 6.8, at room temperature of 22° to 30° C., whereas it dies on beef agar slants, in Cohn's, Fermi's, and Uschinsky's solutions, after 4 months at the same temperatures. Sterile-milk and beef-bouillon cultures kept at 14° are alive after 11 months.

Virulence.—The organism remains virulent for more than a year. Fifteen months after isolating, transfers descended from the original isolation, including a smooth and rough colony, were inoculated into *Gypsophila paniculata* plants. In 7 days the galls were forming and continued to grow rapidly.

Chromogenesis.—On beef agar the color of this organism is at first a light cream that changes to a Naples yellow in a few days. It is much the same in other solid media. Later the color may be mustard yellow or primuline yellow.

MORPHOLOGY

Grown on beef-infusion agar, the *Gypsophila* gall organism is a short rod with rounded ends growing singly, in pairs, and occasionally in chains of four to several elements; in rare cases there have been more than 25. Grown on beef agar for 1 day and stained with carbol fuchsin, the size is 0.5μ to 1.2μ long by 0.3μ to 0.8μ wide. Grown on the same medium for 2 days and stained with gentian violet, the size is 0.4μ to 1.03μ long by 0.2μ to 0.62μ wide. The organism is motile on beef agar and in beef bouillon and its motility was demonstrated by staining with Casares-Gil flagella stain. There are several flagella, all bipolar. Capsules are formed, as was shown by staining young beef agar cultures with Ribbert's capsule stain. The tests for endospores showed none.

STAINING RELATIONS

The organism stains well with gentian violet and carbol fuchsin. It is not acid-fast and is Gram-negative. (Hucker's modification of Gram was used.)

TECHNICAL DESCRIPTION

Bacterium gypsophilae, sp. nov.

A motile rod 0.4μ to 1.2μ long and 0.2μ to 0.8μ wide, with several bipolar flagella; capsules present, no spores; Gram-negative, not acid-fast; colonies on beef-infusion agar are circular, either smooth or rough, yellow, butyrous; clouds beef-infusion bouillon heavily in 18 hours; liquefies gelatin slowly, but not blood serum; is facultative anaerobic; coagulates milk; reduces litmus in 9 to 12 days; grows well in Uschinsky's and Fermi's solutions and unusually well in Cohn's solution; reduces nitrates; produces ammonia and a trace of hydrogen sulphide but no indol; no diastatic action; survives cover-glass drying only 4 days; acid without gas produced with saccharose, dextrose, maltose, mannite, but not lactose, and only a slight amount with glycerin; the optimum temperature for growth is over 30° C., the maximum is 40°, the minimum is 5°; thermal death point is between 52° and 53°; optimum reaction for growth is from pH 6.5 to 6.7, limits of growth from pH 5.1 to 9.0; in beef bouillon and in sterile milk lives 8 months at 22° to 28°, over 11 months at 14°; stains readily with carbol fuchsin and gentian violet; pathogenic to *Gypsophila paniculata* and some of its relatives, producing galls on the crown and root, and cankers on the stem.

COMPARISON WITH BACTERIUM BETICOLA

Because of certain points of resemblance between *Bacterium gypsophilae* and *Bact. beticola* and the lesions caused by them, a study of certain cultural, physiologic, and morphologic characters of these organisms was made. A comparison of these characters is shown in table 2.

NATURAL INFECTION AND CONTROL

The *Gypsophila* gall organism is selective in its host plants, as only related plants have been found susceptible to it; however, there are also related plants which are not susceptible. The limitation of this gall-forming ability differs from that of the crown-gall organism, which produces galls on many unrelated plants. The hosts susceptible to the *Gypsophila* gall organism are *Lychnis chalcedonica* (fig. 3, *F*), *Dianthus plumarius* L. (fig. 3, *D*), *Silene armeria* L. (fig. 3, *B*), and *Saponaria vaccaria* L., which is the weed soapwort (fig. 2, *E* and *F*). This last relative seems to be more susceptible to the organism than *Gypsophila paniculata* itself, for infection begins in 3 to 4 days after inoculation and galls are formed very rapidly. It may be that this weed is the natural host of the *Gypsophila* gall organism and it would be advisable not to allow it to grow in the neighborhood of *Gypsophila* plants. Another relative, *Saponaria ocymoides splendens* Hort., is slow to become infected, as the galls did not begin to form on young plants until nearly 3 weeks after inoculation. The relatives, *Cerastium tomentosum* L., *Tunica saxifraga* Scop., *Spergula pilifera* DC., *Dianthus barbatus* L., which is the common sweet-william (fig. 3, *E*), and the greenhouse carnations are not susceptible. It is an interesting fact that the carnation is not infected by the *Gypsophila* gall organism, for *Bacterium tumefaciens* produces galls thereon quite readily, and occasionally natural *Bact. tumefaciens* galls are found on it. To be quite certain that carnation plants could not be infected by the *Gypsophila* gall organism, inoculations were made at different times of the year and in different growing stages of the plant. Other hosts that did not prove susceptible are sugar beet, tobacco (*Nicotiana tabacum* L.), *Ricinus communis* L., Paris daisy, *Impatiens balsamina*, tomato, geranium, rose (*Rosa* L.), *Bryophyllum pinnatum* Kurz, nasturtium, and two monocotyledons, amaryllis (*Amaryllis* L.) and calla (*Zantedeschia aethiopica* (L.) Spreng.). As stated previously decided swellings but no galls formed on the potato stem.

TABLE 2.—*Comparative cultural, physiologic, and morphologic characters of Bacterium gypsophilae and Bact. beticola*

Character compared	*Bacterium gypsophilae*	*Bacterium beticola*
Colonies in beef agar plates	Circular, none irregular, butyrous, white first day, deep cream to yellow, 4 to 8 mm in diameter in 4 days	Circular, some irregular, viscid, buff colored first day, yellow, 4 to 6 mm in diameter in 4 days
Cohn's solution	Rapid heavy growth	No growth
Liquefaction of gelatin stabs	Begins after 30 days, complete after 4 months	Begins after 7 to 8 days, complete in 20 to 30 days
Hydrogen sulphide production	A trace to none	Rapid and good production
Indol production	None	None
Reduction of nitrates	Nitrates reduced	Nitrates reduced
Reduction of litmus milk	Complete in 9 to 10 days, milk coagulated in 8 to 9 days	Complete in 20 to 30 days, milk coagulated in 10 to 20 days
Gas production	None	None
Acid produced with dextrose, saccharose, glycerin, and mannite	Acidity produced	Acidity produced
Acid produced with lactose	None	None
Ammonia production	Ammonia produced	Ammonia produced
Diastatic action	None	Starch reduced
Relation to oxygen	Facultative anaerobic	Aerobic
Relation to acid and alkali	pH range 5.1 to 9.0	pH range 4.8 to 9.1
Temperature relations	Grows from 5° to 40° C., optimum about 34°, thermal death point 52° to 53°	Grows from 1.5° to 39° C., optimum about 29°, thermal death point 51° to 52°
Survives cover glass drying	4 days	7 days
Color	Ranges from white to yellow	Ranges from buff to yellow
Size	0.4μ to 1.2μ long, 0.2μ to 0.8μ wide	0.6μ to 2μ long, 0.4μ to 0.8μ wide
Gram negative or positive	Negative	Variable
Pathogenicity	Produces galls on *Gypsophila paniculata* but not on sugar beets	Produces galls on sugar beets but not on *Gypsophila paniculata*

The *Gypsophila* gall organism produces cankers on stems of *Gypsophila paniculata*, fair-sized cankers forming in less than 1 month after inoculation, large ones in 3 months (fig. 4, *B*). On *Lychnis chalcedonica* stems the infection is of the typical gall type. *Lychnis* crowns inoculated in November in the greenhouse developed galls, which rotted away during the winter. In the spring, when growth started, new galls formed which became larger than the original ones. This occurred with inoculated *G. paniculata* plants also, although the galls were not so soft and did not disintegrate so easily. The organism, like other organisms that produce galls, is a wound parasite, this one getting into the plant from the soil through imperfect grafting or through cultivation wounds. The disease occurs on lands that have been manured and on those that have not.

The rapid development of galls at the crown produces the death of some of the stems, and if the girdling is complete the death of the entire plant follows. A small gall which has not caused any apparent trouble to the plant may be a decided menace to others later. The method used for propagating *Gypsophila* plants is to graft a desirable variety on seedling roots. If this variety is galled the organism is carried over to the young seedling roots and galls develop. Because of the sensitiveness of the gall organism to weak solutions of mercuric chloride, control measures can be carried on at the time of grafting to reduce the amount of disease occurring in the field. The roots should be dipped in a 1:1,000 mercuric chloride solution for 1½ to 2 minutes to kill the *Gypsophila* gall bacteria that may be on the surface; then with a disinfected knife a well-matched graft should be made and bound with nursery tape.

The sensitiveness of the organism to weak solutions of mercuric chloride was tested out by the poured-plate method. A fresh beef-bouillon transfer was exposed to 1 cc of a 1:1,000 solution of mercuric chloride for various lengths of time and then plates were poured, carrying over a loop of the exposed culture to each agar plate. No *Gypsophila* gall colonies appeared on the plates exposed over 1½ minutes to the mercuric chloride solution. As *Gypsophila paniculata* plants are not very sensitive to a 1:1,000 solution of mercuric chloride they can be treated with it to kill the gall organisms that may be present on the surface. Twenty-five seedling plants 3 to 6 inches tall were soaked in the solution for 1½ minutes, 25 for 3 minutes, 25 for 5 minutes, and 25 for 10 minutes. There was no appreciable injury to the plants from this treatment, even in the 10-minute lot.

In planting out seedlings and grafted plants, in weeding them and in loosening the soil about them during the summer, care should be taken to avoid wounding, for the organism may be present in the soil and may enter the plant through some tiny wound.

SUMMARY

An outbreak of an infectious gall disease occurred in 1932 in an ornamental-plant nursery in the eastern part of the United States. The galls were on the crown and roots of *Gypsophila paniculata*. They were soft and nodular; some were flat and spreading, others globular.

The *Gypsophila* galls when developing do not have the fissures or pockets that occur in developing galls of pocket disease of sugar beets and in those of olive knot. Growth of the galls is favored by hot moist weather, which also favors the disintegration of the old galls, releasing more organisms into the soil. Imperfect grafting and cultivation wounds allow entrance of the pathogene into susceptible tissue. Galls weaken the plants, producing defoliation and death of stems and, where girdling is severe, death of the plants.

An organism, which could be seen readily in sound gall tissue under the microscope, was isolated from the outgrowths and produced galls when inoculated into healthy plants of *Gypsophila paniculata.*

Galls were also produced by inoculation on several species related to *Gypsophila*, but the organism did not produce galls on the sugar beet, which is the host of the pocket disease, nor on such common hosts of the crown-gall organism as the Paris daisy, *Ricinus*, or geranium.

For the pathogene, which is a yellow, polar-flagellate organism apparently unlike any other known gall-producing organism, the name *Bacterium gypsophilae* is proposed. A description of its cultural, physiologic, and morphologic characters is given.

A comparison has been made between the new gall organism and *Bacterium beticola*, the organism causing the pocket disease of sugar beets.

A study has been made of conditions governing the natural occurrence of infection, and methods of controlling the disease are suggested

EXPERIMENTS ON IAROVIZING CORN[1]

By George F. Sprague

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INTRODUCTION

The methods of iarovization advocated by the Russian workers fall into two distinct groups: The low- and the high-temperature treatments. The low-temperature treatments have been known for a long time and are well established experimentally, as is evidenced by the work of Klippart (*3*, *p. 757*),[3] Lysenko (*4*), McKinney and Sando (*6*), and others. The high-temperature treatments are less well established. They have been advocated as a very effective agent in the hastening of sexual maturity in the short-day plants. If the results reported by the Russians are universally confirmed, the process of iarovization might be expected to play a very important role in certain temperate regions. Iarovization of corn in the United States, however, even if effective, does not appear to offer any great commercial possibilities. On the other hand, it might have considerable value in certain physiologic and genetic experiments.

THE PROCESS OF IAROVIZATION

Originally the term "iarovization" (vernalization) was applied only to the low-temperature treatment of winter wheat to induce jointing and hasten sexual maturity. At the present time the term is generally applied to any treatment having as its object the hastening of sexual maturity.

The requirements for different crops differ in the duration of the treatment, the temperature, and the moisture content of the seeds. A summary table is presented by McKinney and Sando (*7*) listing the requirements for a few crops. All of the high-temperature treatments, according to the Russian workers, must take place in darkness.

MATERIALS AND METHODS

The experiments reported in this paper were conducted at the Arlington Experiment Farm, Rosslyn, Va., in 1933. Eight hybrids, 9 inbreds, and 1 variety of corn and 1 strain of teosinte were included. The variety of corn used was Gaspé, one of the earliest known. The hybrids and 7 of the inbreds are adapted to Corn Belt conditions. Of the remaining inbreds one is a derivative of the Garrick variety which is adapted to the South, and the other is a type called "Cuzcoid" because of its resemblance to the varieties from Cuzco, Peru. This last is a simple Mendelian recessive and in the segregating prog-

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[2] The writer acknowledges his indebtedness to H. H. McKinney and W. J. Sando for the use of temperature-control facilities and greenhouse space and for helpful suggestions during the progress of these investigations.

[3] Reference is made by number (italic) to Literature Cited, p 1120.

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enies so far tested has required about twice as long to reach the reproductive stage as its normal sibs. Sexual maturity in this strain has not been hastened by exposure to a 10-hour day.

A further indication of the range of season represented by the inbreds in this test may be obtained from the following comparison. The earliest strain, 616, shed pollen 60 days after planting. Cuzcoid, the latest strain, was killed by frost October 26, 160 days after planting, when less than one-tenth of the plants had tasseled. At this time it had an average of 38 nodes per plant.

All of the seed used was soaked in a 0.5-percent solution of Uspulun for 2 hours and then rinsed. They were then soaked for 9 hours in a weak salt solution of about the same molecular concentration as tap water and were then dried to approximately the moisture content (30 percent) recommended. This method was found to result in much more uniform germination than adding stated quantities of water as recommended by Lysenko (*4*). One complete set of the samples was then placed in light-proof bottles in a constant-temperature chamber and held at 75° F. for 14 days. Duplicates of some of the samples were subjected to the same temperature conditions, but exposed to the normal day or to continuous light (normal day plus artificial illumination). One lot was held at a temperature of 38° F. for the same period. At the end of the 14-day period all of the samples were planted in the field. In those samples in which germination had progressed the farthest the radicles were approximately 5 to 8 mm and the plumules 5 mm long. Molds, particularly *Penicillium*, occurred in some seed lots, but no visibly infected seeds were planted.

The iarovized material was hill-checked in some cases in comparison with sprouted seed, and in other cases with dry seed of the same sort. In some instances, the difference due to the slight hastening of emergence because of the sprouted condition of the iarovized seed persisted and was reflected in a slightly earlier tasseling and silking (table 2, items 3 and 6). This should not be a serious source of error in experiments of this kind, as a slight advantage from this cause would be insignificant as compared with the marked acceleration which must result from iarovization if the method is to be commercially practical.

Dates of germination, pollen shedding, and silking were recorded for the plants in all perfect hills. In addition, percentage of germination, number of nodes, plant heights, and plant yields were obtained in most cases. All of the data reported are based on comparisons between plants of the same strain growing in the same hill.

EXPERIMENTAL RESULTS

The effect of the treatments on field germination and on emergence of the inbreds is shown in table 1. In every case iarovization resulted in a marked reduction in germination and in 13 of the 14 cases delayed emergence. The germination of the iarovized seed was so poor and seedling mortality following germination so great that no adequate data on maturity are available. For the three strains A, 324, and 540, which had the highest germination percentage and for which meager data are available, there was no indication of a hastened maturity following the treatments.

TABLE 1.—*Effects of iarovization on field germination and emergence in 14 inbred strains of corn*

Inbred strain no.	Condition of check	Germination: Iarovized	Germination: Check	Mean difference between iarovized and check seed lots in time to emergence [a]
		Percent	*Percent*	*Days*
616	Sprouted	35.0	97.5	1.4
A	do	70.0	97.5	1.3
324	do	75.0	100.0	.9
420	do	55.0	95.0	2.0
420 [b]	do	32.5	97.5	1.7
420 [c]	do	7.5	97.5	2.5
461	do	22.5	97.5	1.8
461 [b]	do	25.0	95.0	1.7
461 [c]	do	40.0	100.0	2.0
461	Dry	20.0	97.5	1.7
540	Sprouted	80.0	97.5	.3
119-11 b	do	22.5	92.5	2.0
207 37	do	35.0	72.5	−.4
Cuzcoid	do	54.0	83.0	+.5

[a] Positive differences indicate the iarovized lot emerged later than the check. In the column headed emergence a positive difference indicates that the iarovized lot required more days to emerge than the control.

[b] Iarovized in light (day only).

[c] Iarovized in light (continuous).

With the more vigorous material, which includes the hybrids and the variety Gaspé, there was a slight but consistent reduction in germination as a result of iarovization, as shown in table 2. The time required for germination shows no consistent differences resulting from the treatments. Only three of the mean differences are significant. In two of these, iarovization appears to have had an accelerating effect, but in both the check seed was dry and ungerminated. In one instance there is a significant retardation. In this paper, differences have been considered statistically significant when P is 0.02 or less. Such differences are italicized in table 2.

In 8 of the 15 comparisons, plants from iarovized seed shed pollen significantly before the checks, but in only 5 cases was there a significant difference in silking. This is in accord with general observations that pollen shedding is influenced to a greater extent by environment than is silking. There is a fairly high positive correlation between the days required for emergence and for pollen shedding. Substantially the same degree of correlation exists in both the iarovized and control lots. It should perhaps be emphasized that while in several cases hastening of sexual maturity is statistically significant, in no case is the acceleration of any significance agronomically.

The numbers of nodes visible at maturity were fewer in the plants from iarovized seed in all of the 11 comparisons involving iarovized *v.* noniarovized seed and of which counts were made. Only 7 of the 11 differences are statistically significant, but 11 deviations of like sign would be expected only once in 2,048 trials as a result of sampling error alone. Counting nodes visible at maturity is not satisfactory where the absolute number is wanted. In the present case, however, the interest lies in the relative difference between paired plants of successive hills, and the visible number of nodes is just as satisfactory a basis for comparison as the absolute number.

TABLE 2.—*The effects of iarovization on various agronomic characters in 8 hybrids and 1 variety of corn*

[Differences for which P is less than 0.02 are italicized]

Hybrid or variety	Condition of check	Field germination		Mean differences between iarovized and control plantings in—[a]						
				Time to—			Nodes	Plant height	Ears	Weight of shelled grain per plant
		Iarovized	Check	Emergence	Pollen shedding	Silking				
		Pct.	*Pct.*	*Days*	*Days*	*Days*	*No.*	*Inches*	*No*	*Grams*
A×325	Sprouted	87.5	95.0	0.8	−0.7	0.3	*−1.9*	*−9*	−0.1	−14.8
A×164	Dry	95.0	100.0	−.4	*−3.0*	*−1.9*	*−1.8*	*−7*	.0	*−48.8*
A×164 [b]	do	100.0	100.0	*−1.0*	*−1.7*	*−1.6*	−.3	*−9*	−.1	−18.7
A×164	Sprouted	92.5	100.0	.0	*−2.0*	*−1.2*	*−1.6*	*−5*	−.1	*−44.5*
420×164	Dry	72.5	95.0	.0	−.6	−.4	*−1.1*	*−4*	−.6	*−40.0*
420×164 [b]	do	95.0	97.5	*−1.0*	*−2.1*	*−1.3*	−.3	−3	−.1	−8.9
420×164	Sprouted	85.0	87.5	.6	.6	.7	*−1.0*	−3	−.3	−28.1
420×4 8	do	97.5	100.0	0	*−1.0*	−.5	*−1.2*	−3	.0	*−38.0*
325×420	do	100.0	100.0	−.1	*−1.7*	−.9	−.8	−3	.0	*−30.1*
325×420 [c]	do	95.0	100.0	.0	*−2.7*	*−1.9*	*−1.0*	−3	.0	−1.9
540×164	do	95.0	100.0	.6	.1	.2	−.8	−4	−.1	−23.9
L×317	do	97.5	100.0	−.5	*−1.6*	−1.0	−.7	−3	.0	−10.3
317×461	do	100.0	97.5	.4	−.3	−.2	−.7	1	−.3	−23.7
Gaspé	do	95.0	100.0	*1.5*	.7	.6				
Do.[d]	do	95.0	97.5	.6	−.3	−.5				

[a] Positive difference indicates iarovized lot exceeds check.
[b] Sprouted, not iarovized
[c] Iarovized, continuous light.
[d] Iarovized at 38° F

There appears to be no consistent relationship between the reduction in number of nodes and plant height as a result of the treatment. In some strains the iarovized plants exhibit a significant reduction in number of nodes and yet are not significantly shorter than their controls.

Where any differences exist in number of ears, the iarovized material always has the lower number. None of the differences, however, is significant.

All of the iarovized material exhibits a reduced yield of shelled grain per plant, only five of the comparisons, however, being significant. The reductions in yield indicated in table 2 as being significant represent a reduction of approximately 15 to 30 percent. It is worthy of mention that the comparison exhibiting the least reduction in yield was significantly earlier in pollen shedding and silking than its controls and was iarovized under continuous light.

In the variety Gaspé iarovization at 75° F. or at 38° F. for a 14-day period was ineffective in hastening sexual maturity.

DARKNESS REQUIREMENT

According to the theory advanced by Lysenko (*4*), short-day plants require light for processes of growth and the absence of light (darkness) for the initiation of reproduction. The necessary darkness may be supplied continuously during the early stages of the plant's development or as periods alternating with light, as in day and night, during a greater portion of the plant's development. Iarovization carried on in the dark is presumed to be effective, in the case of short-day plants, because it satisfies the plant's requirement for darkness. With this requirement satisfied, the plants are able to benefit from the long days, and hastened sexual maturity results.

The theory of a "darkness requirement" for the initiation of reproduction in corn, at least for some varieties of the temperate regions, is not in agreement with several facts. Corn has been grown in the greenhouse at the Arlington Farm during two winters under continuous illumination (normal day plus artificial illumination) without any material delay in the onset of flowering or maturity. In the winter of 1932 33 four inbred strains representing a considerable portion of the seasonal range of Corn Belt varieties were grown under continuous illumination from the dry seed to maturity. The plants were perfectly normal in their vegetative development and seasonal maturity.

As a further test for the necessity of a dark period, the same strains of corn were grown during the summer, one set of plants being exposed to the normal day, and a second set to continuous illumination (normal day plus artificial illumination). The results are presented in table 3.

TABLE 3.—*Effect of day length on sexual maturity in 4 inbred strains of corn*

Inbred strain no	Period from planting till pollen shedding when grown with a day length of—		
	12 hours	16 hours	24 hours
	Days	*Days*	*Days*
616	--	52	60
A	--	55	61
228-4-8	61	62	69
461	----	60	66

Continuous illumination resulted in approximately a week's delay in pollen shedding. For the one strain grown also with a 12-hour day, there was no significant difference in earliness between the 12-hour and 16-hour photoperiods.

The classification of corn as a short-day plant does not appear to be based on adequate experimental evidence. It is true that the work of Garner and Allard (*2*), Emerson (*1*), and McClelland (*5*) has shown that some varieties respond to a short day (10 to 12 hours of light). However, all of the varieties used by these investigators were tropical or semitropical sorts naturally adapted to short-day conditions. Varieties which are adapted to Corn Belt or more northern conditions and which normally bloom during a long day (15 to 18 hours of daylight) have not been adequately studied. In three strains grown in the greenhouse, augmenting the winter day (11 to 13 hours) by artificial illumination for 4.5 or 8.5 hours has not resulted in a significantly delayed sexual maturity.

Teosinte has been shown by Emerson (*1*) to respond to a short day. It was thought that a comparison of the effectiveness of darkness supplied to this plant during the iarovization process and as a daily photoperiod would be instructive. Three lots of teosinte were planted May 19, one of them having been iarovized. The iarovized lot and one of the others were exposed to the natural day. There was no significant difference in sexual maturity of these two lots, both showing tassels September 16 and shedding pollen October 3. The second noniarovized lot was exposed to a 10-hour day. It showed tassels June 6 and shed pollen June 10, having been exposed to fewer hours of darkness (328) during this period than had the iarovized lot (336 hours) during the period of iarovization. It seems clear from this that length of day is much more important in determining the time of sexual maturity than is any darkness requirement that teosinte may possess (fig. 1).

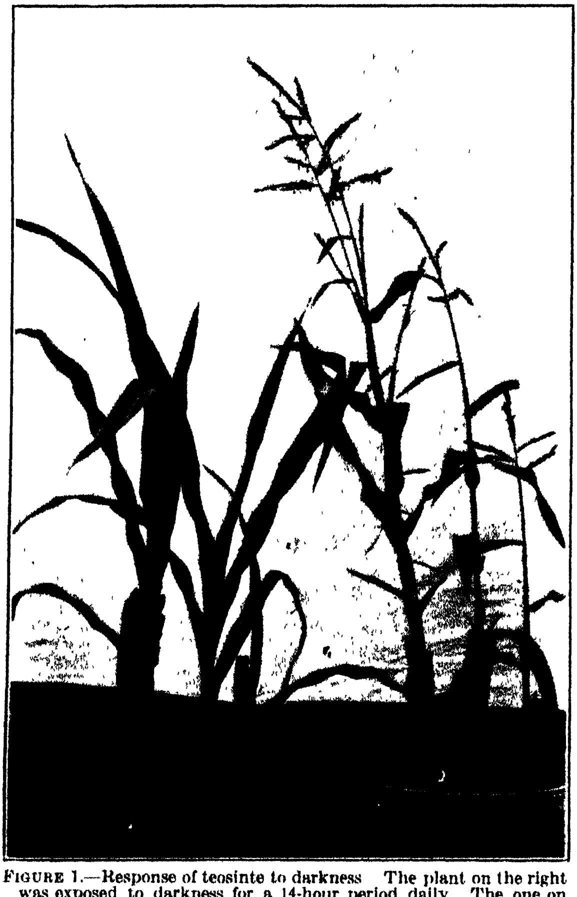

FIGURE 1.—Response of teosinte to darkness The plant on the right was exposed to darkness for a 14-hour period daily. The one on the left was exposed to darkness continuously for 14 days followed by exposure to darkness for an 8- to 9-hour period daily. Photographed June 19, 31 days after planting.

McKinney and Sando (8) have shown that after the iarovization treatment the attainment of sexual maturity in winter wheat is greatly influenced by day length. To determine whether a similar condition exists in short-day crops, a second set of material, common millet and the corn hybrid A×164, were iarovized at 80° F. at the moisture contents and for the periods recommended, one lot of seed of each crop in continuous darkness and a duplicate lot in continuous light (normal day plus artificial illumination). Following iarovization, the various lots, including dry and germinated checks, were grown in pots under a 16-hour and a 24-hour day.

The treatment given the millet was ineffective in hastening sexual maturity under either light treatment. The corn plants receiving the 16-hour day responded approximately as they had done in the field. For the plants grown under continuous illumination, iarovization with continuous light was superior to iarovization with continuous darkness in promoting early flowering, and both lots from iarovized seed were earlier than those from either the germinated or dry checks. The results are presented in table 4. There is nothing in the results obtained in these experiments with corn and teosinte to lend support to the darkness-requirement theory of Lysenko.

TABLE 4.—*Influence of day length on the attainment of sexual maturity in corn*

Photoperiod (hours)	Period required to attain sexual maturity under indicated treatments			
	Iarovized		Control	
	Continuous dark	Continuous light	Germinated	Dry
	Days	*Days*	*Days*	*Days*
16	64	61	65	67
24	76	71	80	81

DISCUSSION

The iarovization of certain corn hybrids resulted in a statistically significant hastening of sexual maturity. The difference, however, was so slight as to be of no importance agronomically. The general reduction in germination and vigor associated with the iarovization treatment appears to be a serious limitation of the method. In this respect these results depart rather drastically from those reported by the Russian investigators. The reason for the failure of agreement with their results is not entirely clear.

The Russians emphasize the fact that varieties differ markedly in their iarovization requirements. It is possible that all of the strains used in these tests require special conditions during iarovization, though this does not seem likely.

The experiments of McKinney and Sando (*8*) and the results reported here (table 4) are in agreement in indicating that the day length following iarovization has an important effect on the reaction. The plants in the present studies (tables 1 and 2) were grown under a daily photoperiod of approximately 15 hours, and those in the Russian work presumably under a longer daily photoperiod. It seems probable that at least part of the difference between the results in the two places may be ascribed to the day length under which the plants were grown. The claims for the necessity of darkness during the iarovization process, however, are not substantiated by either the field or greenhouse tests.

SUMMARY

Iarovization of corn, as practiced, consistently reduced the percentage of germination. It also resulted in a general reduction in the number of visible nodes, in plant height, number of ears, and weight of shelled grain per plant.

Pollen shedding and silking were significantly accelerated by iarovization in some strains but not to an extent to be of any agronomic importance.

There was no evidence of a darkness requirement for corn. Several strains of corn were grown to maturity under continuous light. Iarovization in continuous light was just as effective as that in continuous darkness.

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THE MICROBIAL DECOMPOSITION OF SUCCESSIVE CUTTINGS OF ALFALFA HAY UNDER AEROBIC CONDITIONS[1]

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INTRODUCTION

The present investigation was undertaken to determine whether there is any difference in the decomposition of successive cuttings of alfalfa hay aerobically fermented by soil micro-organisms.

A review of the literature gives little information regarding the influence of chemical composition on the microbial decomposition of successive cuttings of alfalfa hay.

Falck and Haag (*1*)[2] concluded from their studies that in the microbiological decomposition of plant materials two distinct processes take place, namely, destruction and corrosion. The effect of destruction is to decompose the cellulose and pentosans, the lignins being very little affected. Corrosion, on the other hand, causes slow decomposition of both lignin and cellulose.

Starkey (*3, pp. 293–294*) stated:

> When the decomposition of organic matter is measured by the amount of CO_2 produced, it should be kept in mind that the CO_2 is not the only product formed from the carbon of the organic matter * * * since various intermediate products may be formed * * *. In general, however, the incomplete decomposition products of some organisms are further attacked by others and sooner or later appear as CO_2. * * * The CO_2 produced from soils should, therefore, give a reliable although not an absolute index of the decomposition of organic matter.

Waksman and Tenney (*6*) found that when a comparison is made of the rapidity of decomposition of a plant which has been harvested at different stages of growth, different results are obtained. The more mature the plant is the less readily does it decompose. Their analysis showed that a third of all the constituents of the young rye plant, on a dry basis, consisted of water-soluble substances, including considerable quantities of sugars and amino acids. The young plant contained, on a dry basis, 2.5 percent of nitrogen, 7.7 of ash, 16.6 of pentosan, 18.06 of cellulose, and 9.9 percent of lignin. With the advance in the age of the plant, there was a rapid decrease in the nitrogen and in the fat and ash content and a gradual increase in the cellulose, pentosan, and lignin content. There was considerable decrease in the amount of water-soluble constituents. The mature plant (exclusive of the grain and roots) contained 0.24 percent of nitrogen, 22.9 percent of pentosan, 36.3 percent of cellulose, 19.8 percent of lignin, and 9.9 percent of water-soluble substances.

EXPERIMENTAL METHODS

In order to determine differences in the microbial decomposition of successive cuttings of alfalfa hay, two experiments, each in duplicate, were conducted. Three cuttings of hay were collected from each of

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[2] Reference is made by number (italic) to Literature Cited, p. 1126.

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the 1931 and 1932 crops under similar conditions but from different fields. The hay used in the first experiment (1931 crop) was collected from a field on the United States Department of Agriculture Experiment Farm at Beltsville, Md., on which heavy manuring and intensive cultivation methods were used. The hay used in the second experiment (1932 crop) was collected from a privately owned field on which only ordinary fertilizing and cultivation methods were used. As Van der Spuy and Stead (*4*) have shown by analyses of plants at various stages of growth that the nutritive value of lucerne hay is highest when the hay is cut at the 10-percent stage of flowering, and also that the highest yield of hay is obtained by cutting at this stage of growth, the alfalfa hay for each experiment was collected when most of the plants were in partial bloom. Each cutting was obtained from the same area in each field in order that environmental growth factors would be the same Immediately after cutting, the material was sun-dried for several days and then passed through a grinder

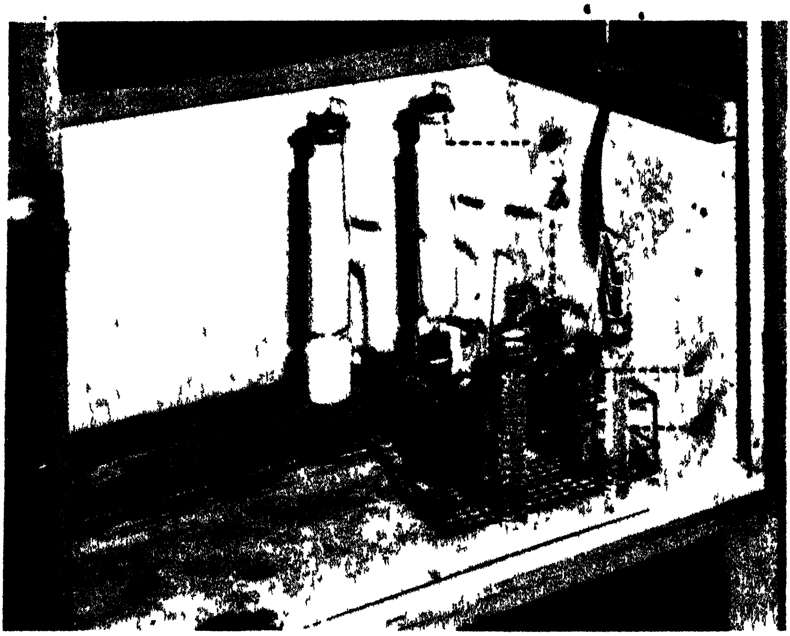

FIGURE 1 Aerating apparatus for the determination of carbon dioxide in fermenting plant materials *a*, Soda lime tower *b*, humidifier *c* fermentation tube *d* spiral absorber

In the first experiment, two 10-gram samples (calculated on a dry basis) of each of the three cuttings were inoculated with sufficient soil infusion to give a total moisture content of 43 percent In the second experiment, two 6.5-gram samples of each cutting were similarly prepared The soil infusion used in each experiment was prepared by mixing 100 grams of soil, obtained from the same area in the field from which each cutting of hay was obtained, with 150 cubic centimeters of water and filtering through sterile cotton. The filtrate was thoroughly shaken and used as the inoculum for each of the three cuttings of hay.

The inoculated hay samples were placed in large test tubes fitted with aeration tubes extending to the bottoms, connected to an aerating apparatus, and incubated at 30° C. for 30 days. Each aerating apparatus (fig. 1) consisted of a soda lime tower, *a*, containing wet pieces of sponge for moistening the air before it was passed through the hay sample; a fermentation tube, *c*, containing the hay sample; and a spiral absorbing tube, *d*, containing potassium hydroxide (*2*) to absorb the carbon dioxide produced from the fermenting hay.

Air was passed through the apparatus from a pressure line connected to a large "bleeder" bottle and then to the soda lime tower. The "bleeder" bottle was necessary to reduce the pressure and also to adjust the air flow at any desired rate. It consisted of a 15-liter bottle equipped with inlet and outlet tubes and a stopcock through which the excess pressure was released. The air flow was regulated at such a rate that the gas bubbles passing through the spiral tube containing the potassium hydroxide came in contact with the absorbing liquid long enough to allow for complete absorption. This rate of flow was found to be approximately 2 liters of air per hour. Determinations were made to check the efficiency of the soda lime towers and spiral absorbers. Daily titrations of the potassium hydroxide were made, and the quantities of carbon dioxide evolved from the fermenting hay were determined and recorded as milligrams of carbon.

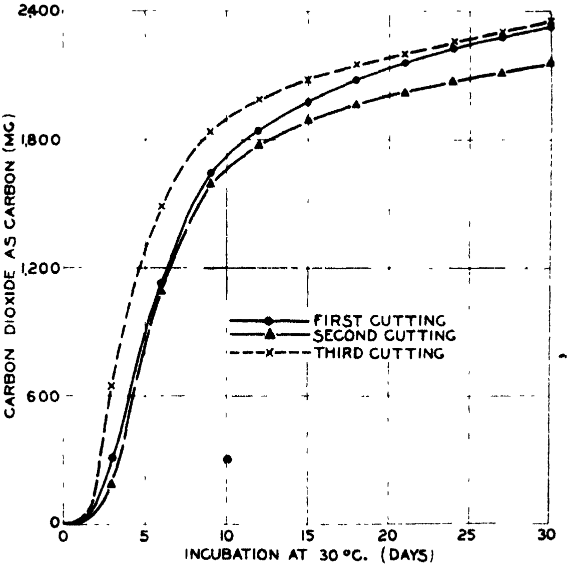

FIGURE 2.—Total carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop.

Oven-dried samples from the three cuttings of the 1931 crop were analyzed for the important chemical fractions by the modified method of Waksman and Tenney (*7*). Owing to limited facilities, the hemicelluloses, cellulose, and lignin were not directly determined but were recorded as the fraction soluble in 2-percent HCl, the fraction soluble in 80-percent H_2SO_4, and the residue, respectively. At the end of the fermentation period, the material remaining was dried to constant weight at 105° C. From the results obtained by the difference in weight before and after fermentation the percentage loss due to microbial action was determined. The oven-dried material was then analyzed as previously mentioned. Each determination was calculated on the basis of the original weight of material used, thereby showing the actual percentage loss of each fracton caused by the microbial decomposition. The chemical analyses on both the unfermented and fermented hays were repeated several times in duplicate.

RESULTS

Results on the total quantities of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 and 1932 crops are shown in figures 2 and 3. In both experiments (figs. 2 and 3), the third cutting showed the greatest decomposition, the first cutting was next in order, and the second cutting showed the least decomposition. Results on the daily titrations of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop are shown in figure 4.

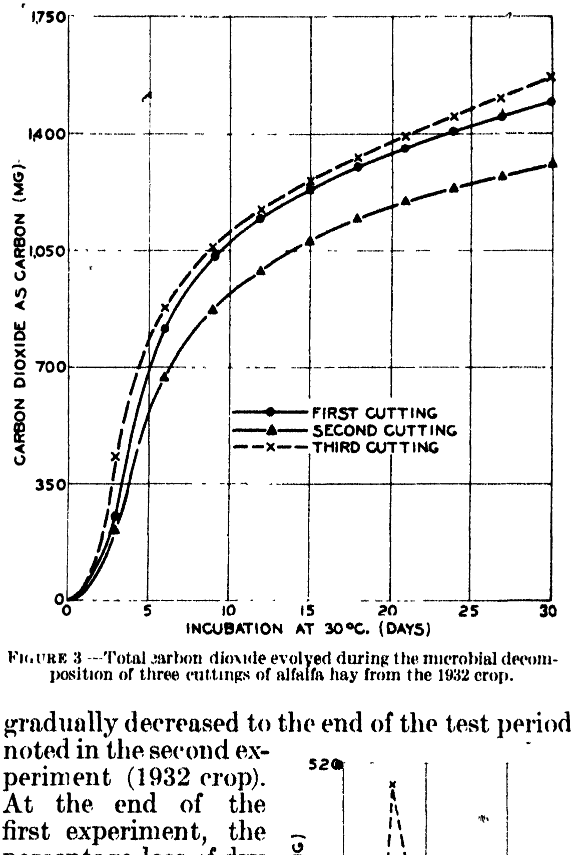

FIGURE 3.—Total carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1932 crop.

It will be noted that the greatest variation in the rate of decomposition of the three cuttings of hay occurred during the first 10 to 12 days of the fermentation, after which it gradually decreased to the end of the test period. Similar results were noted in the second experiment (1932 crop). At the end of the first experiment, the percentage loss of dry matter due to microbial decomposition was 41.3 for the third cutting, 39.2 for the first, and 35.8 for the second. In the second experiment, the percentage loss was 43.6 for the third cutting, 41.4 for the first, and 37.3 for the second.

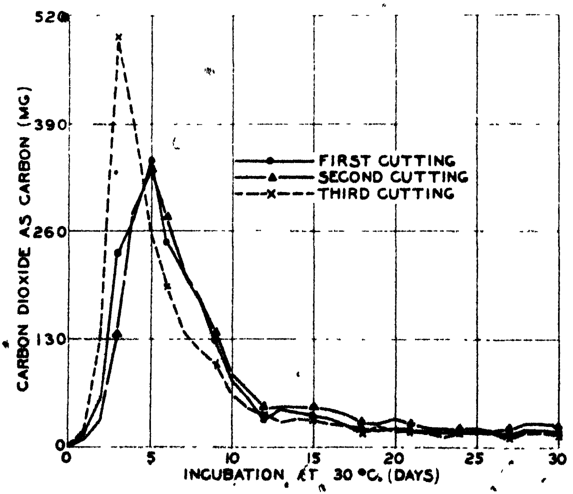

FIGURE 4.—Daily quantities of carbon dioxide evolved during the microbial decomposition of three cuttings of alfalfa hay from the 1931 crop.

Chemical analyses were made on oven-dried samples from the three cuttings of hay used in the first experiment before and after microbial decomposition. The results shown in table 1 were included to give the approximate chemical composition of each cutting of hay before fermentation and similar data after fermentation, showing the percent loss in each of the fractions. It is noted that in the larger fractions (cold water, 2-percent HCl, and 80-percent H_2SO_4), although the percentages in the original hays were approximately the same in each cutting, the percentage losses after fermentation varied. According to Waksman and Tenney (*5*), the various fractions were composed of the following constituents: (1) The ether fraction contained fats and waxes; (2) the cold-water fraction contained simple carbohydrates, various amino acids, peptides, and soluble minerals; (3) the hot-water fraction contained starches, pectins, certain hexosans, and various nitrogenous compounds; (4) the 2-percent HCl fraction contained hemicelluloses and protein; (5) the 80-percent H_2SO_4 fraction contained cellulose and protein; and (6) the residue contained lignin, protein, and ash.

TABLE 1.—*Percentage composition (by fractions containing various constituents) of successive cuttings of alfalfa hay from the 1931 crop before and after microbial decomposition, and percentage loss of each fraction*

[On moisture-free basis]

Chemical fraction	First cutting: Original material	First cutting: After 30 days decomposition: Calculated on original weight	First cutting: After 30 days decomposition: Loss	Second cutting: Original material	Second cutting: After 30 days decomposition: Calculated on original weight	Second cutting: After 30 days decomposition: Loss	Third cutting: Original material	Third cutting: After 30 days decomposition: Calculated on original weight	Third cutting: After 30 days decomposition: Loss
Ether	2.0	1.7	15.0	3.0	2.7	10.0	2.2	1.7	22.7
Cold water	24.6	10.3	58.1	24.2	13.7	43.3	26.7	13.2	50.5
Hot water	5.1	3.2	37.2	4.2	4.0	4.7	4.6	2.8	39.1
2-percent HCl	28.6	17.3	39.5	29.2	15.8	45.8	30.3	17.4	42.5
80-percent H_2SO_4	23.3	15.4	33.9	22.2	14.0	36.9	19.4	9.8	49.4
Residue	14.0	11.0	21.4	13.6	11.9	12	13.3	12.0	9.7
Protein insoluble in cold H_2O [a]	12.3	7.3	40.6	13.2	7.2	45.4	15.5	8.6	44.5
Total nitrogen [a]	2.5	1.5	40.0	3.1	1.6	48.3	3.1	1.8	41.9
Ash [a]	9.7	9.7	0	9.7	9.7	0	9.6	9.6	0

[a] Analyses carried out on separate samples.

Determination of the quantity of the individual constituents in each fraction was not made. In the larger fractions the variations in the losses the three cuttings were probably due to differences in the amounts of easily fermentable substances.

The types of micro-organisms in the fermenting hay greatly influenced the decompositions. The ground hay was very porous, hence it favored the rapid development of fungi, especially actinomycetes, which are capable of rapidly decomposing organic matter. Slimy masses of bacteria were also present in the fermenting hay although a more compact medium would have favored their development. The microbial decompositions were probably accelerated by the high nitrogen content in each cutting.

SUMMARY

A study was made of the influence of chemical composition on the microbial decomposition of successive cuttings of alfalfa hay. Data on the microbial decomposition of each cutting were obtained by measuring the daily evolution of carbon dioxide during a test period of 30 days, and by chemical analyses before and after fermentation.

Of the three cuttings of hay tested from the 1931 and 1932 crops, the third cutting underwent the greatest decomposition, the first cutting was next in order, and the second cutting showed the least decomposition.

The greatest variation in the rate of decomposition occurred during the first 10 to 12 days of the fermentations, after which it gradually decreased to the end of the experiment.

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PRESS FLUID FROM HEATED BEEF MUSCLE[1]

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INTRODUCTION

Juiciness, which is dependent upon the fluid content of muscle, is an important factor in palatability of meat, and quality is closely associated with palatability. Howe[2] describes juiciness in meat as its readily expressible liquid.

A grading chart with descriptive terms for scoring factors of palatability in meat has been developed by the cooking committee of the cooperative meat investigations committee.[3] This chart grades quantity of juice in meat as "very juicy", "moderately juicy", "slightly dry", "dry", "very dry", and "extremely dry." Quality of juice is graded as "very rich", "rich", "moderately rich", "slightly rich", "perceptible", "slightly perceptible." Grading the quantity and quality of juice in meat by such a method depends upon individual differences and standards of the judge. More accurate methods of determining both quantity and quality of juice are necessary for scientific experimentation in order that data of different investigators may be definitely compared.

The literature describes no definite method for pressing the fluid content from muscle, and little data can be found dealing with this pressed fluid.

This investigation was undertaken to develop a laboratory method for pressing out muscle fluid, by which different meat samples might be given a relative rating for quantity of press fluid or juice.

The purposes of this study were (1) to develop an apparatus for the removal of press fluid from roasted beef muscle; (2) to standardize methods for determining the percentage of press fluid and the ratio between press fluid and dry matter, and for obtaining press fluid for chemical analysis; (3) to use the standardized methods for studying press fluid in two beef muscles, psoas major, and biceps femoris; and (4) to compare the moisture, ether extract, and nitrogen contents of the press fluid from roasted beef muscle under pressure for two periods of 5 minutes and 20 minutes.

APPARATUS AND MATERIALS

The apparatus used in this study is called a "pressometer" (fig. 1). The term "press fluid" is used in preference to "juice" since in meat studies juiciness is graded by the individual's reaction when meat is eaten and probably includes not only fluids which are present in meat, but depends also upon the flow of saliva stimu-

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[1] Received for publication Jan. 18, 1934, issued July 1934. Contribution from Minnesota Agricultural Experiment Station, Scientific Journal Series, Paper No 1216 The authors gratefully acknowledge the technical assistance of Christian Dane and George Steinacher

[2] HOWE, P. E. RELATION OF COOKING TO THE STUDY OF THE QUALITY AND PALATABILITY OF MEAT. Jour. Home Econ. 19. 8-15. 1927.

[3] ALEXANDER, L. M, CLARK, N G, and HOWE, P. E. METHODS OF COOKING AND TESTING MEAT FOR PALATABILITY. Revised February 1933 Supplement to National Project Cooperative Meat Investigations. U.S. Dept. Agr, Bur. Home Econ and Bur. Anim Indus 36 pp, illus 1933 [Mimeographed]

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June 15, 1934
Key no. Minn.-85

lated by meat extractives. Therefore, "press fluid" is used to designate the fluid consisting of moisture plus the soluble material plus the colloidal fraction that is pressed from muscle by the pressometer.

The pressometer (fig. 1) consists of a heavy cast-iron base with an attached motor. The muscle sample is wrapped in filter cloth (fig. 3) and placed in a brass tray (fig. 2) which is fitted into a grooved platform in the apparatus. Starting the motor, the platform with tray is driven upward by means of a large screw until it reaches a stationary plunger, the bottom of its base having the same dimensions as the inside of the tray. A 25-pound brass weight, which is suspended from the lever bar, exerts pressure on the sample. The pressure may be varied from 250 to 500 pounds by placing the weight at different points on the lever. When the desired pressure has been attained, the lever arm is lifted, breaking the current, and automatically arresting an increase in pressure. The actual time of pressure is checked by an automatic timer or stop watch. To carry the platform downward, the switch is reversed.

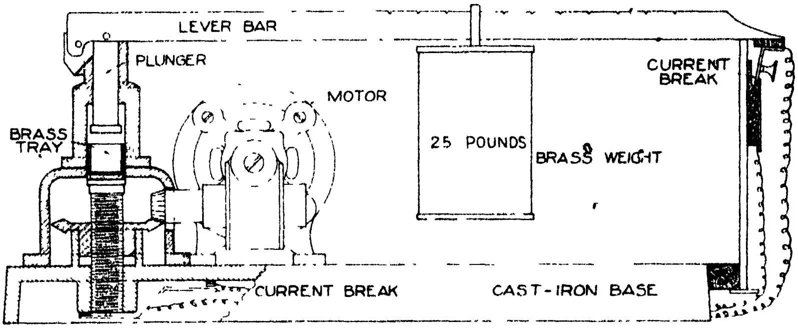

FIGURE 1.—Cross section of the pressometer, giving detail of working mechanism.

Samples were taken from beef roasts averaging 1½ pounds, from the left and right sides of the same animal. The roasts were stored in an electric refrigerator at 4° to 6° C. until used for cooking.

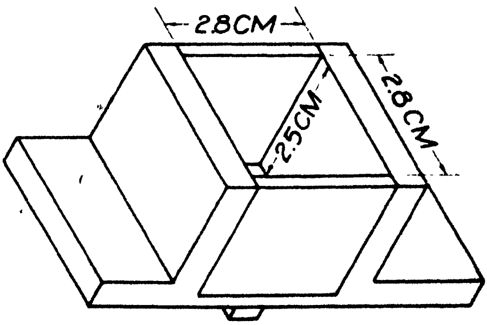

FIGURE 2.—Brass tray for holding muscle sample.

The rump was removed from the wholesale round by cutting parallel and ventral to the pelvic bone (aitch bone). On the medial side (inside or top round) at this point, there are two large muscles, the posterior one being the semimembranosus muscle, immediately in front of which lies the adductor muscle from which samples were taken for the removal of press fluid.

Roasts were prepared according to the method given in the quality tests officially accepted by the cooking committee of the cooperative meat investigation committee,[4] but were not seared. The exterior fat was removed, and the meat wiped with a damp cloth; it was then weighed, and all data for computing losses were recorded. The roast was tied with a string so that it was cylindrical in shape. The center of the bulb of a weighed, straight thermometer was inserted in the center of the roast. The meat was placed on a heavy wire rack, the rack being 1.25 cm from the bottom of the pan, in a weighed sheet-iron roasting pan (23.8 by 18.4 by 6 cm). The pan was set crosswise in the oven which was preheated to 125° C. The meat was roasted until the thermometer registered 60°.

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[4] ALEXANDER, L. M., CLARK, N. G., and HOWE, P. E. See footnote 3.

STANDARDIZATION OF METHOD FOR USING PRESSOMETER

The first work on pressing muscle was done by placing the meat in a tray (2.5 by 2.7 by 0.8 cm) with a sieve bottom, the press fluid being collected in a tray placed directly under it. Because of the small size of the sample with which it was necessary to work, much of the fluid was lost either by evaporation or by adherence to to the tray, and small quantities of the solid part of the muscle were pressed through the sieve. Later, a tray with a slight metal elevation (height 0.3 cm) in the center was used, thus forming a groove around the edge, where the fluid could collect. This method, also, proved to be inadequate since it was difficult to press all of the fluid from the edges of the muscle and to remove it from the tray; therefore, it was decided to wrap the sample in a piece of cloth.

After trying different kinds, sizes, and shapes of cloth for wrapping the muscle sample, it was decided that unsized filter cloth, cut cross shape (fig. 3), gave the best results. The crosses were cut from the filter cloth after it had been boiled 10 minutes in distilled water and then dried.

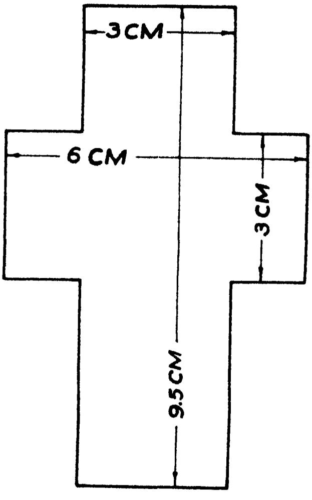

FIGURE 3.—Cloth cross for wrapping muscle sample

For sampling, a slice of meat was cut from the center of the roast with a sharp thin-bladed knife. A mechanical gage was used for determining the exact thickness. In order to obtain the most desirable thickness for cutting the meat, slices of different thicknesses (2.5, 1.87, and 1.25 cm) were tried. Those cut 2.5 cm thick were too large and showed that all the press fluid was not absorbed by the cloth. The press fluid from the 1.87-cm and 1.25-cm slices was absorbed by the cloth. It was decided to use the 1.87-cm slice in order to obtain more easily a sufficient quantity of fluid for chemical analysis.

A brief study was made of metal borers of different sizes and shapes. Two round borers (one, 1.27 cm in diameter, and the other, 2.4 cm in diameter) and a square borer (1 cm square) were used. The round borers were more easily used than the square one. The round borer 1.27 cm (fig. 4) in diameter was chosen, as the small sample had a more uniform structure of muscle fibers than the larger one.

Experimental work was carried out using different pressures for 10 minutes. The average [5] of press fluid pressed out by 112 pounds pressure was 43.90 percent; by 168 pounds pressure, 49.73; by 226.5 pounds pressure, 50.26; by 250 pounds pressure, 56.84; and by 500 pounds pressure, 58.61 percent. The data obtained showed that increased pressure yields more press fluid. Two hundred and fifty pounds pressure was selected because it was found that this pressure could be used also with chicken muscle, which is short fibered. Some of the solids of this tender muscle were pressed into the cloth when 500 pounds pressure was used.

To determine the optimum length of time for the removal of press fluid, four periods were used: 5, 10, 15, and 20 minutes. The mean press fluid for seven samples was as follows: 5 minutes, 49.00 percent; 10 minutes, 49.28 percent; 15 minutes, 50.64 percent; and 20 minutes, 50.66 percent.

Since there was not a great variation in the mean percentage of press fluid obtained from these different periods, the 10-minute period was used, as it allowed sufficient time for weighing samples and less time for evaporation.

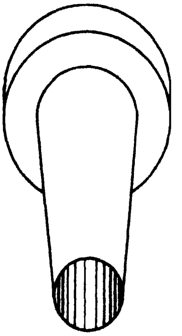

FIGURE 4.—Borer, 7.6 cm in length and 1.27 cm in diameter, used for cutting muscle sample.

The average press fluid in nine muscle samples was 49.93 percent, based on the difference between the weight of the original sample and the pressed muscle. The average press fluid in the cloth from the same samples was 48.98 percent, based on the weight of the original dry cloth and the cloth moistened with the pressed fluid. The slightly lower percentage of fluid in the cloth than in the muscle was probably due to a faster evaporation in the cloth.

To compare the quantity of press fluid from roasts of different temperature, samples were cut (1) at the time the roasts were taken from the oven, (2) 2 hours after taking from the oven, (3) 3½ hours after taking from the oven. The mean press fluid from roasts at approximately 55.5° C. was 49.64 percent; at 23.3°, 49.74 percent; and at 21.1°, 49.52 percent. From the mean percentages of press fluid obtained from samples of roasts at these different temperatures, it can be seen that there was not an appreciable difference in the total quantity. It seemed evident that all of the small samples of muscle were about the same temperature at the actual time of pressing. To prove this, thermocouples were used to obtain the temperature of the small samples from the roasts of varying temperatures, and it was found that by the time the sample was cut, weighed, and wrapped in cloth, the muscle had almost reached room temperature (21°). Since there was so little difference in the quantity of press fluid, all roasts were cooled to 40° before sampling.

METHODS SELECTED FOR OBTAINING PRESS FLUID FROM ROASTED BEEF MUSCLE

From the preliminary work the following were chosen as desirable methods for obtaining press fluid from roasted beef muscle and were used in succeeding experiments.

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[5] The percentages press fluid for 112, 168, and 227.5 pounds pressure were obtained by using an unimproved pressometer.

A slice 1.87 cm thick was cut from the center of the roast by means of a sharp thin-bladed knife, using a mechanical gage for determining the thickness of the slice. Three adjacent samples were cut from the center of the slice with a round borer 1.27 cm in diameter (fig. 4). Each sample was transferred to a numbered, previously weighed, aluminum dish containing a piece of dry, weighed, unsized, shrunken filter cloth that was cut cross shape. The dish with cloth and muscle sample was then weighed. The sample was carefully wrapped and placed in the tray (fig. 2), which was inserted in the pressometer and allowed to remain for 10 minutes at a pressure of 250 pounds. The muscle sample and the cloth were removed from the tray and placed in separate, dried, weighed aluminum dishes and weighed; samples were kept in the desiccator until ready for weighing. Rapid work was necessary from the time the sample was cut to the last weighing, so as to avoid evaporation losses. Forceps were used for all handling.

The percentage of press fluid in the muscle was found by dividing the weight of the press fluid by the weight of the muscle sample before pressing. The weight of the press fluid was found by subtracting the weight of the pressed muscle sample from the weight of the unpressed sample.

METHOD USED IN OBTAINING RATIO OF PRESS FLUID TO DRY MATTER FROM HEATED MUSCLE

The method for obtaining the quantity of press fluid from heated muscle, as previously explained, was followed. The two aluminum dishes with weighed samples of pressed muscle in one and the cloth containing the press fluid in the other were then dried in a Freas vacuum oven at 65° C. The ratio of press fluid to dry matter was calculated by dividing the weight of the press fluid by the weight of the dry matter. The dry matter includes both that in the residual muscle and that adhering to the cloth.

METHOD USED FOR OBTAINING PRESS FLUID FOR CHEMICAL ANALYSIS

For chemical analysis of the press fluid, samples of muscle were cut from the center slices of the roast.

The sample was set in the center of a weighed, cross-shaped filter cloth, wrapped, and placed in the pressometer and allowed to remain for 5 minutes at a pressure of 250 pounds; the pressed muscle was then discarded and the process was repeated, using the same piece of cloth with two other muscle samples. The cloth was removed to a weighed corked bottle and placed in the desiccator. When four pieces of cloth were similarly filled with press fluid, the bottle and cloth were weighed and the amount of press fluid was calculated. This quantity of press fluid (5½ g or more) was sufficient to determine the ether extract, nitrogen, and moisture content. The corked sample bottle, containing the filter cloth with pressed fluid, was kept in a cold room (14° to 15° C.) until needed for analysis.

The sample, after being prepared for analysis, was cut in pieces approximately one-fourth inch square and was divided into five aliquot portions, each being placed in a weighed aluminum dish. The covered dishes were allowed to stand at room temperature for

15 minutes, then weighed. Moisture, ether extract, and nitrogen were determined by the official methods of analysis of the Association of Official Agricultural Chemists.[5]

EXPERIMENTAL DATA FROM USE OF THE PRESSOMETER

The three methods of experimentation were used for comparing (1) the press fluid in two beef muscles, one tender (psoas major) and one less tender (biceps femoris), and (2) the moisture, ether extract, and nitrogen in the press fluid from roasted adductor muscle when the samples were kept under pressure for two periods, 5 minutes and 20 minutes.

RATIO OF PRESS FLUID TO DRY MATTER IN ROASTED BEEF

Table 1 presents the mean grams of fluid per gram of dry matter from a tender muscle (the psoas major) of roast beef, and a less tender muscle (the biceps femoris). The mean grams of fluid per gram of dry matter (1.847 for biceps femoris and 1.823 for psoas major) did not vary significantly, as determined by means of the t test [6]; t is 0.226 and P is between 0.90 and 0.80 which means that in 80 to 90 cases out of 100 this difference is due to chance.

TABLE 1. *Mean[a] grams of press fluid per gram of dry matter in the muscles, biceps femoris and psoas major, from roasted beef*

Roast no	Biceps femoris muscle	Roast no	Psoas major muscle
1	1.620	9	1.815
2	2.076	10	1.660
3	1.583	11	1.336
4	1.946	12	2.160
5	1.903	13	2.013
6	1.796	14	1.596
7	2.100	15	1.926
8	1.753	16	2.080
Mean	1.847	Mean	1.823

[a] From 3 samples from center slice of roast

MOISTURE, ETHER EXTRACT, AND NITROGEN IN PRESS FLUID FROM ROASTED BEEF WHEN SAMPLES WERE UNDER PRESSURE FOR PERIODS OF 5 AND 20 MINUTES

The percentage of moisture in press fluid from the roasted adductor muscle of beef under pressure for periods of 5 and 20 minutes is given in table 2

The mean percentage of moisture in the press fluid when the samples remained under pressure for 5 minutes was 89.974, and for 20 minutes 88.757, showing slightly less moisture after it had been under pressure for 20 than for 5 minutes. This slight difference may be due to greater evaporation when the sample remained under pressure for the longer time; t is 1.640 and P is between 0.20 and 0.10, which means that in 10 to 20 cases out of 100 this difference is due to chance. These data indicate that duration of pressing is not likely to affect the percentage of moisture in press fluid.

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[5] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS OFFICIAL AND TENTATIVE METHODS OF ANALYSIS Compiled by the committee on editing methods of analysis. Ed 3, 593 pp, illus Washington, D.C., 1930

[6] FISHER, R A STATISTICAL METHODS FOR RESEARCH WORKERS Ed 4, rev and enl, 307 pp, illus Edinburgh and London. 1932.

TABLE 2. *Percentage of moisture, ether extract, and nitrogen in press fluid from roasted adductor beef muscle under pressure for 5-minute and for 20-minute periods*

5 minutes		20 minutes	
Roast no	Moisture	Roast no	Moisture
	MOISTURE		
18	90 96	19	88 16
20	84 37	21	89 52
22	90 32	23	89 05
24	90 44	25	90 67
26	90 39	27	90 72
28	89 28	29	90 54
30	89 53	31	87 94
32	91 15	33	90 38
34	90 49	35	88 52
36	86 80	37	90 01
38	90 71	39	91 08
40	89 24	41	86 49
42	82 25	43	78 83
44	89 71	45	90 38
Mean	89 97	Mean	88 757
	ETHER EXTRACT		
18	1 81	19	3 14
20	2 39	21	2 82
22	2 53	23	2 86
24	2 00	25	2 48
26	1 05	27	2 16
28	3 65	29	3 07
30	2 87	31	1 87
32	1 61	33	97
34	1 70	35	1 86
36	3 24	37	2 61
38	1 74	39	73
40	2 82	41	7 18
42	7 32	43	7 07
44	1 26	45	1 87
Mean	2 571	Mean	2 906
	NITROGEN		
18	1 010	19	1 020
20	980	21	950
22	984	23	965
24	1 021	25	946
26	9[illegible]0	27	927
28	938	29	942
30	997	31	985
32	975	33	906
34	1 005	35	925
36	1 002	37	950
38	888	39	866
40	1 000	41	957
42	963	43	964
44	1 010	45	951
Mean	982	Mean	947

The quantity of fat in press fluid may be an indication of the quality of press fluid or juice in meat In order to determine whether time of pressing had any effect on the quantity of fat, the ether extract obtained from press fluid of roasted adductor beef muscle under pressure for 5 minutes was compared with that obtained under 20 minutes' pressure (table 2) In the case of ether extract the 5-minute period gave a mean percentage of 2 571, and the 20-minute period one of 2 906, *t* being 0.914 and *P* between 0.40 and 0 30, indicating that the difference is not significant.[7] A longer time under pressure is not likely to influence the final percentage of ether extract obtained from the press fluid, since muscle fat solidifies when cool.

The mean percentage of nitrogen (table 2) in the press fluid from the roasted muscle was found to be significantly higher after a 5-minute expression period than after a 20-minute period, being 0.982 percent for the former and 0.947 percent for the latter. The t test applied to this difference gave $t = 4.843$, and P is less than 0.01. One might assume that more nitrogen would be obtained in press fluid when the longer period is used, because of the possible pressing out of colloidal material into the cloth. A possible explanation of the fact that the greater percentage of nitrogen appears during the 5-minute period than during the 20-minute period, is that the soluble nitrogen is pressed out at the beginning of the period. Gortner, Lawrence, and Harris[8] in their work on plant tissue, state that in some instances the fluid extracted by continuous pressing, without rearrangement of the tissue mass, may become less and less concentrated. This was found to be true in a series of extractions from cabbage leaves.

SUMMARY

An apparatus called the "pressometer", developed to press fluid from heated beef muscle, is described.

Methods are explained for using the pressometer, for determining the percentage of press fluid and the ratio of press fluid to dry matter in heated beef muscle, and for obtaining press fluid from heated beef muscle for chemical analysis.

From this study the following observations may be made on the basis of statistical analysis:

The mean percentages of ether extract and moisture in the press fluid from the adductor muscle from roasted beef, did not vary significantly when samples were under pressure for 5 and for 20-minute periods.

The mean percentages of nitrogen in the press fluid from adductor muscle from roasted beef varied significantly when samples were under pressure for 5 and for 20-minute periods, the greater percentage of nitrogen appearing during the 5-minute period.

The mean percentages of press fluid from the muscles psoas major and biceps femoris from roasted beef did not vary significantly when the grams of press fluid per dram of dry matter were compared.

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[7] Fisher, R. A. See footnote 6.

[8] Gortner, R. A., Lawrence, J. V., and Harris, J. A. THE EXTRACTION OF SAP FROM PLANT TISSUES BY PRESSURE. Biochem. Bull. 5: 139-142. 1916.

CARBON DIOXIDE FORMATION BY CLEAN AND SCABBY POTATOES [1]

By B. F. Lutman [2]

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INTRODUCTION

Losses in weight of potato tubers in storage are due to both transpiration and respiration, but much the greater losses are due to the former. The checking of such losses by the tubers is largely through the skin. The skins of scabby potatoes are altered considerably by a parasitic growth which stimulates the formation of a loose and very permeable covering. Such tubers lose weight reapidly after removal from the soil. The gradual healing of the abnormal skin areas after the tubers have been in storage for some time tends to check these heavy initial losses and to restore the tubers almost to the same condition as healthy ones.

PREVIOUS WORK

The relation of respiration to loss of weight in potatoes, apples, carrots, and other fruits and vegetables has been discussed in considerable detail by various authors. Especially important in this connection is the work of Appleman, Kimbrough, and Smith (*1*)[3] on the physiological shrinkage of potatoes in storage, and that of Kimbrough (*5*) on the respiration of potatoes during storage and transportation. Some interesting work on the internal gases of potato tubers has been reported by Magness (*7*). A detailed account of the relative losses in weight of clean and scabby tubers is given in an earlier report by the present writer (*6*).

The method used by all these investigators except the last mentioned is to pass carbon-dioxide-free air through a vessel containing a weighed lot of potatoes. This air sweeps out the carbon dioxide formed by the tubers. The air with its accumulated carbon dioxide is then passed through a solution such as barium hydroxide. A titration of this solution at the conclusion of the run gives the amount of carbon dioxide evolved.

The method used by the author (*6*) was the sampling method, the determination of the percentage of carbon dioxide in the 10-cc sample being determined by the Haldane apparatus. By this method the respiration of 10 pounds of potatoes kept under bell jars between October 4 and March 29 was only 2.471 for clean tubers or 3.757 for scabby ones. The temperature during much of this storage period was only 2° C. Although at the beginning of the season it has been 12°, it dropped rapidly to about 3° to 4°. The total respiration loss of the tubers kept by Kimbrough (*5*) is not given, but from his graph the

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[1] Received for publication Nov. 3, 1933; issued July 1934.

[2] The writer is indebted to John B Vander, assistant chemist, Vermont Agricultural Experiment Station, for the chemical work involved in the storage studies of the clean and scabby potatoes

[3] Reference is made by number (italic) to Literature Cited, p 1143.

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loss between October 1 and February 7 (129 days) for a kilogram of tubers kept at 22° C. (71.6° F.) appears to be about 17. The respiration at 40° of Green Mountain tubers, according to Wright (9), was in milligrams per kilogram-hour on November 7, 5.11; on January 17, 3.57; and on March 24, 2.93. At these rates the total loss per kilogram would be for the 5 storage months, November to March, inclusive, about 15 g.

In a recent publication Smith (8) reviewed much of the literature and added extensive data on the effect of temperature, humidity, injury, depth of layer, etc., on potatoes in storage. Ordinary storage cellars were used in some of the experiments, and the loss in weight from respiration during a 7-month period ranged from 0.40 to 0.67 percent of the total weight of the tubers. The loss from respiration of mechanically injured tubers, while large at first, dropped rapidly until it was no greater than that of uninjured ones. The depth of the potatoes in the bin had little effect on the carbon dioxide formation, although it was somewhat higher in the bottom layers than in the top ones. The difference was noticeable only at the end of the storage season when sprouting was probably starting. Smith does not discuss the relative merits of the methods used for respiration determination.

PURPOSE OF THE WORK

The purpose of the experiments here reported was primarily to ascertain the loss by respiration of scabby potatoes as compared with that of clean ones after various periods in storage, and to compare the results obtained by the aspirator method with those obtained by the sampling one.

METHODS

Four lots of tubers as nearly alike as possible in size and number were placed under bell jars. Each lot weighed approximately 1,000 g. Two of the lots were composed of clean tubers and two of scabby tubers. The jars contained 8 liters of air after the tubers had been placed in them. To prevent the growth of mold, the tubers were immersed for 1 hour in a weak formaldehyde solution before they were placed in the jars, and fresh tubers were substituted if decay appeared. The jars were kept in a basement room the temperature of which ranged from 13° to 15° C.

The amount of carbon dioxide evolved was measured by (1) the aspirator method, and (2) the sampling or Haldane method. The amount of carbon dioxide formed in a jar of clean and in one of scabby tubers was determined by each method.

When the aspirator method was used laboratory air, freed from carbon dioxide and moisture (fig. 1), was passed through the bell jar containing the tubers and then into a small Erlenmeyer flask containing 100 cc of 0.1650 normal barium hydroxide solution. After the air in the bell jar had been passed for 24 hours through this solution, it was titrated with 2/10 N oxalic acid and the amount of carbon dioxide computed.

An analysis of the air inside bell jars containing clean or scabby tubers was made daily by the use of a Haldane apparatus during the early part of the storage season and usually at intervals of 2 days during the late fall. The tubers were left under the jars for periods of

5 to 7 days during the winter, the carbon dioxide being allowed to accumulate in the jar. The air in the bell jars was thoroughly mixed each time by the aid of a celluloid fan operated from outside to insure a homogeneous mixture.

The intercellular gas of the tubers used in the experiments was removed from a number of the lots. The method of extraction was that described by Magness (7), and the determination of carbon dioxide and oxygen was made by a modified Henderson apparatus (fig. 2). The modification consisted in the use of a 3-cc calibrated tube instead of the usual 10-cc tube. Three cubic centimeters of gas was usually all that could readily be obtained. Eight plugs, 1 or 2 from each tuber, were placed in the mercury. The modified Henderson apparatus is not so accurate as the Haldane, but it is probably not more than 1 percent in error.

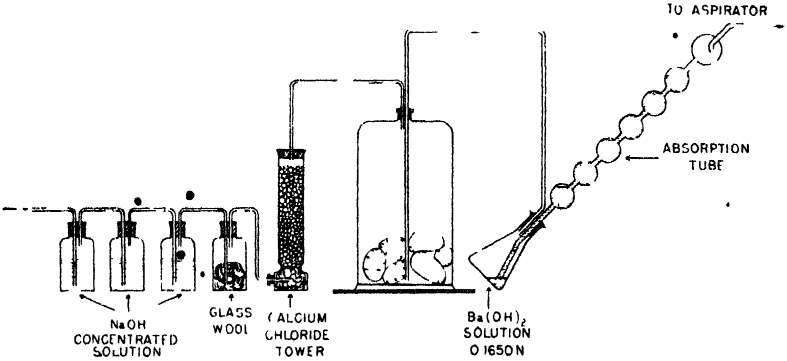

FIGURE 1.—Diagram of apparatus used for obtaining the carbon dioxide production by the use of a continuous current of carbon dioxide free air.

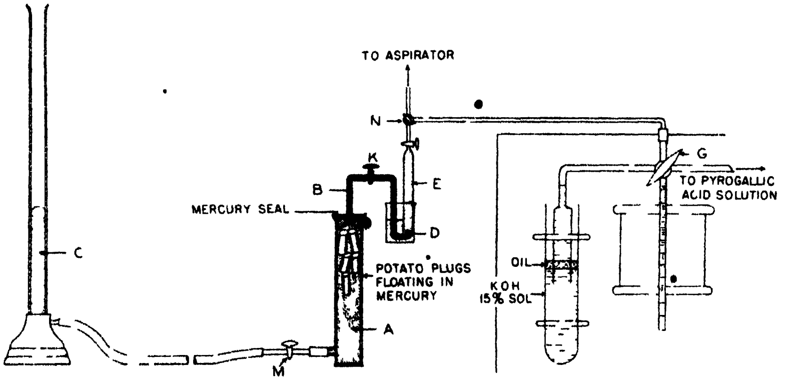

FIGURE 2.—Diagram of apparatus used for the extraction of internal gases from the potato tuber and for the determination of the percentage of carbon dioxide and oxygen in the 3-cc tube employed in the modified Henderson apparatus. *A*, Jar filled with mercury and potato plugs; *B*, tube filled with mercury; *C*, jar filled with mercury, connected by heavy rubber tubing with jar *A*, controlled by stop cock *M*; jar *C* can be raised and lowered; *D*, beaker filled with mercury in which is placed the gas-collecting tube *E*, controlled by stopcock *K*; *N*, stopcock from collection tube *E*, to aspirator or to CO_2 and O_2 apparatus; *G*, three-way stopcock to calibrated CO_2 apparatus immediately below or to pyrogallic acid solution on right but not shown in the figure.

To determine the amount of carbon dioxide that would accumulate in air-tight potato bins, two large cylindrical, galvanized iron tanks, each 5½ feet high, were filled with clean or scabby potatoes. Before the potatoes were placed in the bins, glass tubes with a bore of about

2 mm (barometer) were placed in each so as to tap the air between the tubers at the 5-, 4-, 3-, 2-, and 1-foot levels. The upper end of each tube was closed by a pinchcock. Samples of the storage air at the various levels could be drawn through the tubes without disturbing the other air or the tubers.

DATA

LOSS OF CARBON DIOXIDE FROM CLEAN AND SCABBY TUBERS

Four lots of tubers were carried through the storage season under bell jars. These tubers were changed every 2 to 3 weeks from a supply kept under the same conditions. Two jars contained clean tubers and two contained scabby ones. The respiration in a clean and in a scabby jar was measured by the absorption-aspirator method and similar lots were measured by the sampling method. The experiments were continued from October 11, 1930, to March 19, 1931, a total storage period of 159 days. A summary of the results, presented in tables 1 and 2, shows that the general effect of scabbing was to increase the loss of carbon dioxide except during the last month or so of storage. The loss as determined by the absorption method was almost twice as great as that by the sampling method.

TABLE 1.—*Carbon dioxide production (milligrams) by clean and scabby potatoes during the storage season, as determined by the absorption and sampling methods*

Month	Average production of CO_2 per kilogram-hour				Average production of CO_2 per kilogram per day			
	Absorption method		Sampling method		Absorption method		Sampling method	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
October	8.8	10.0	6.7	8.7	211.2	240.0	160.8	208.8
November	4.1	6.0	2.7	4.0	98.4	144.0	64.8	96.0
December	3.3	5.[illegible]	1.6	2.2	79.2	141.6	38.4	52.8
January	4.2	4.9	2.1	2.4	100.8	117.6	50.4	57.6
February	5.5	5.1	2.7	2.8	132.0	122.4	64.8	67.2
March	9.3	6.7	4.0	3.5	223.2	160.8	96.0	84.0

TABLE 2.—*Total carbon dioxide production (grams per kilogram) by clean and scabby potatoes, by the month or part of the month, and also for the entire storage season, as determined by the absorption and sampling methods*

Month	Absorption method		Sampling method	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
October (20 days)	4.224	4.800	3.216	4.176
November	2.952	4.320	1.944	2.880
December	2.455	4.390	1.190	1.637
January	3.125	3.645	1.562	1.785
February	3.696	3.427	1.814	1.881
March (19 days)	4.241	3.055	1.824	1.596
Total (159 days)	20.693	23.637	11.550	13.955

The results are a reflection of the pathology of the scabby tubers and are an interesting addition to the physiology of abnormal growths. The loss of carbon dioxide was relatively heavy from the scabby tubers during the early part of the season (October to February), but by February the scab lesions had healed so thoroughly that the skins of the scabby tubers were as impermeable as those of the clean ones. During March the scabby tubers should apparently have had an even better protection against the loss of carbon dioxide than the clean ones. The fact that the clean tubers produced more carbon dioxide late in the season was due to their earlier germination, as table 3 shows.

TABLE 3. *Carbon dioxide production (milligrams per kilogram-hour) by clean and scabby potatoes during 3 periods in March, as determined by the absorption and sampling methods*

Period	Absorption method		Sampling method	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
Mar 1 to Mar 5	5 8	6 0	2 8	
Mar 9 to Mar 13 (sprouting in clean tubers begun)	10 2	6 3	3 3	
Mar 15 to Mar 19 (sprouting in clean and some in scabby tubers advanced)			6. 4	

Scab spots delay germination from 1 to 2 weeks, and during that time the carbon dioxide production is greater from clean tubers with their large sprouts.

COMPARISON OF RESULTS OBTAINED BY THE TWO METHODS

As compared with the sampling method the absorption method gave a uniformly higher production of carbon dioxide from both clean and scabby tubers (table 4). The conditions under which the comparisons were made should be noted. When the absorption method is used all the air from the bell jar is swept out by the air current passing over the tubers; when the sampling method is used the air over the tubers remains the same and the carbon dioxide produced remains in it. However, the amount of carbon dioxide in the jars never approached 2 percent, the limit of determination by the Haldane apparatus.

TABLE 4. *Daily carbon dioxide production (milligrams per kilogram-hour) by clean and scabby potatoes for the days after the tubers had been aired and then replaced under the bell jars, or other tubers substituted, as determined by the absorption and sampling methods*

Date	Absorption method		Sampling method	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
Dec 9	2. 9	6 1	2 0	4. 2
10	3 6	6 0	1 5	1 9
11	5 1	6 1	1 5	2 2
12	3 4	6. 0	1 4	2. 0
13	3 3	5 9	1. 3	1 8
Jan. 7	3. 7	5. 3	3. 1	3. 5
8			1. 8	2. 0
[illegible]	3 9	5. 0	1 8	1. 8
9			1 7	1 7
29	4. 6	4 3	3 3	3 6
30			2 3	2 5
31	4. 5	4 1	2. 0	2. 3
Feb. 1	--	--	1. 8	2. 2

The amount obtained with the Haldane apparatus the first day after the tubers were placed under the bell jars was similar to that obtained by the other method, but the drop was abrupt after that time and the amount remained relatively small. The tubers used were stored in the same room as the bell jars and had been kept there for at least 1 week before they were used.

Every period after the changing of the tubers showed a similar slump in the rate of respiration as indicated by the Haldane apparatus. The carbon dioxide in the other bell jars must have had the tubers as its source. The internal, intercellular gases percolate out through the lenticels from the inside of the tubers. With this point in mind, a measurement of the internal gases of the tubers under each of the jars was made at intervals after a series of readings had been taken for carbon dioxide formation (tables 5 and 6).

and scabby potatoes on days upon which the internal gases were also analyzed, as determined by the absorption and sampling methods

Date	Absorption method		Sampling method	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
Nov. 5	5.8	7.8	4.3	8.1
Nov. 27	2.3	4.1	1.3	1.5
Dec. 18	3.4	6.3	1.3	2.0
Jan. 17	4.0	5.1	2.1	2.2
Feb. 16	4.4	4.2	2.3	2.4
Mar. 4	6.1	6.6	1.2	1.0
Mar. 21	11.0	8.2	8.6	8.4

TABLE 6.—*Percentage of carbon dioxide and of oxygen in the internal gases of stored potato tubers after the completion of the respiration tests*

Date	After absorption method				After sampling method			
	CO_2		O_2		CO_2		O_2	
	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes	Clean potatoes	Scabby potatoes
Nov. 5	35.6	28.6	7.8	12.0	40.0	56.1	11.0	6.9
Nov. 27	35.5	33.1	8.6	9.0	42.6	52.8	8.0	5.7
Dec 18	29.8	28.3	6.8	10.7	35.9	46.1	11.7	9.7
Jan. 17	32.5	36.5	8.1	6.9	35.3	43.7	9.5	8.7
Feb. 16	28.2	32.9	10.4	11.0	37.1	42.7	8.6	6.2
Mar. 4	22.7	55.3	7.0	10.1	33.8	54.8	5.2	9.9
Mar 21	45.6	58.3	3.4	8.3	55.1	60.1	4.1	5.9
Average	32.84	39.00	7.44	9.71	39.97	50.90	8.30	7.57

When determinations were made by the sampling method the amount of carbon dioxide remaining in the internal gases of the tubers was higher in all except one instance (when it was about the same) than it was when the absorption method was used (table 6), indicating that in the passage of the air through the bell jar for 24 hours, a considerable percentage of the internal carbon dioxide found its way out of the tuber. Determinations by the absorption method showed that during November and December, before the scab lesions had completely healed, the scabby tubers allowed more carbon dioxide to pass out than the clean ones, i.e., the percentage remaining within them was smaller. In January, however, and the months following, the percentage remaining inside the scabby tubers was higher than in the clean ones.

AMOUNT OF CARBON DIOXIDE REQUIRED TO CHECK RESPIRATION

The total accumulated carbon dioxide under the bell jars used for sampling never approached 2 percent. Trials made to determine the percentage necessary to check respiration, not to prevent it entirely, indicated that 7 to 9 percent was sufficient and that the acidity of the juice changed from about pH 6.2 to pH 5.8. Such a high percentage of carbon dioxide could probably never develop in a storage bin. The 7 percent carbon dioxide slowed down respiration to a minimum, but at least 16 percent of carbon dioxide was required to prevent it entirely. Kidd (*4*) found that 20 percent would not only stop respiration but would also prevent sprouting.

AMOUNT OF CARBON DIOXIDE ACCUMULATING AT BOTTOM OF BINS

In order to determine the quantity of carbon dioxide that would accumulate in a bin, potatoes were stored in two large open-topped cylinders, clean tubers in one and scabby tubers in the other. Before the potatoes were put into the cylinders, rods of barometer glass were so suspended that air could be withdrawn from depths of 1, 2, 3, 4, and 5 feet. When these experiments were started about March 15, 1931, the storage temperature was approximately 38° to 40° F. Samples were taken weekly, but the percentages of carbon dioxide remained the same as that of the surrounding air until April 15. By that time sprouting was common on both clean and scabby tubers, and the temperature rose to 50° to 52° and the percentage of carbon dioxide in the bottom of the cans rose to 0.7 percent. In 1933 the experiment was repeated, starting February 9 and continuing until May 3. The readings from clean and scabby potatoes were approximately the same, 0.4 percent at 1 foot, 1.3 percent at 2 feet, 1.8 percent at 3 feet, 1.7 percent at 4 feet, and 1.8 percent at 5 feet.

The percentages which appear in the later readings are higher than those of Smith (*8*), but Smith's data are from tubers stored in bins that were not necessarily airtight, whereas the data recorded in this work were from tubers stored in cans. The top layers even in a can of potatoes, however, interpose only a small obstacle to the outward diffusion of gases formed inside the can. If such a formation is slow, as occurs from potatoes stored at low temperatures, the diffusion may keep pace with the formation in a bin that is only 5 feet in depth. The holes between the tubers, as was found by actual measurement, represent 17.7 percent of the surface area of the large potatoes and 14.8 percent that of the small ones. According to Brown and Escombe (*2*), if 11.34 percent of a septum area is composed of holes, the diffusion is 60 percent that of an open vessel. The irregular shape of the holes undoubtedly added to their diffusion efficiency as compared with circular holes. The 15 to 18 percent area of the interstices between the tubers would accordingly mean a diffusion equal probably to at least 70 percent of that of an open vessel.

Other factors, such as shape of pores and depth to which they are embedded, velocity of wind stirring, and the percentage of any gas in the storage chamber, affect the rate of gas diffusion (*3*), but these factors were not investigated in the present work as the area of a tortuous passage from the bottom of a container filled with potatoes would be difficult to determine.

DISCUSSION

Respiration was always less under the bell jars tested by the Haldane apparatus than in those through which the carbon-dioxide-free air was passed. This may be explained by the diffusion of gases through the pores of an impervious membrane. The skin of the potato tuber is the membrane broken by the openings which are the lenticels. The chamber containing the CO_2 is the potato.

Brown and Escombe (*2*) have shown that around each pore an area of gas collects; very dense at the pore, gradually becoming more diluted as the distance from the pore increases. This is their so-called "shell" of gas. Exactly the same shell of CO_2 must occur over each pore, i.e., over each lenticel of the tuber. Let us see how these shells of gas behave under the two treatments.

In the aspirator method, when air, free of CO_2, passes over a lenticel, at the entrance to which the percentage of CO_2 is approximately, let us say 28.6 percent, the shell of carbon dioxide is swept away, leaving a very sharp gradient between the internal gas of the tuber and the air rushing by the opening. The CO_2 is densest at the center of the tuber away from these pores. The CO_2 rushes out of the tuber, is swept along and measured as part of the respiration. This lowers the amount of CO_2 remaining inside the tuber, as shown in table 6. The apparent respiration CO_2 by this method therefore includes (1) the carbon dioxide in the jar; (2) the carbon dioxide surrounding the pores, i.e., the shells; and (3) part of the carbon dioxide which is normally within the tuber but which rushes out through the pores when air is swept over them for 24 hours.

In the sampling method, the air inside the bell jars is still. Obviously a larger shell of diluted CO_2 can be formed over the pore. The preparations are made for taking the sample. The air is stirred by a celluloid fan, breaking up the shells around the pores and distributing the CO_2 throughout the air of the bell jar so as to make a homogeneous gaseous mixture. The 10-cc sample is then taken immediately, the whole process involving only a few minutes. Some of the internal CO_2 rushes out through the pore, but the amount is negligible because the operation is of such short duration. The apparent respiration by the sampling method, therefore, includes CO^2 from only two sources: (1) that which is in the bell jar entirely free from the tubers; and (2) the shells of CO_2 distributed throughout the jar by the celluloid fan.

A comparison of tables 5 and 6 will show the correctness of the data on which the foregoing explanation is based. A discussion of the physical phase may be found in Brown and Escombe's paper (*2*) and the effect of currents of air in Huber's (*3*).

The aspirator method would most properly be used for studies on apple storage; the sampling method would be better for potatoes and root crops. Neither of these methods would give absolute correctness. The same conclusion on potato storage may be drawn from this work that Smith (*8*) drew from his, namely, that carbon dioxide will never collect in sufficient quantity to make ventilation in a storage cellar necessary.

SUMMARY

The respiration records of four lots of potatoes were studied through a storage period of 159 days. Two of these lots were composed of clean tubers and two of scabby tubers.

The respiration rate was obtained (1) on one lot each of clean and scabby tubers by passing carbon-dioxide-free air through the bell jars in which they were kept, the carbon dioxide being absorbed by an alkaline solution; and (2) by withdrawing 10-cc samples from one lot each of the clean and scabby tubers and determining the percentage of carbon dioxide by the use of a Haldane apparatus.

During the first month of the storage period the respiration rates of the scabby tubers were much higher than those of the clean tubers as the cork layers under the scab lesions were not so impervious to gases as the skin of the uninjured ones. The rates tended to become equalized during January and February, but after sprouting began in March the clean tubers respired more than the scabby ones, as sprouting was delayed for some time (7 to 10 days) by the scab lesions.

The respiration rates obtained by passing carbon-dioxide-free air through bell jars containing tubers were uniformly higher than those obtained by taking samples of the air in bell jars containing tubers. The reason suggested for the higher respiration rates obtained by the absorption method is that the flow of air through the jar removes the carbon dioxide not only from around the tubers but also from the gases held in the intercellular spaces. It is also suggested that the checking of respiration from tubers under the bell jars used in the Haldane apparatus tests may have been due to "shells" of carbon-dioxide-laden air around the opening of the lenticels. These shells are not disturbed in the air of the bell jar and check the outward diffusion of carbon dioxide.

The small amount of carbon dioxide which had accumulated inside the bell jars used for the Haldane apparatus tests was not sufficient to check respiration, 7 to 9 percent being required to check it.

The amount of carbon dioxide which appeared at the bottom of a special storage bin in which the tubers were 5 feet in depth was practically that of the surrounding air during the storage season and never rose until the tubers began to sprout, when as high as 1.8 percent occurred at the bottom with a temperature of about 50° F. This diffusion of carbon dioxide from the surface of potato bins follows the laws of the diffusion of gases through pores. With large tubers 17.7 percent of the surface was pore area, and this surface offered little resistance to the diffusion of the carbon dioxide formed at the bottom.

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